Leukemia (2008) 22, 1503–1518
© 2008 Macmillan Publishers Limited All rights reserved 0887-6924/08 $30.00
www.nature.com/leu

REVIEW

Epigenetic plasticity of chromatin in embryonic and hematopoietic stem/progenitor cells: therapeutic potential of cell reprogramming

G Zardo[1,2], G Cimino[1] and C Nervi[2,3]

[1]Department of Cellular Biotechnologies and Hematology, 'La Sapienza' University of Rome, Rome, Italy; [2]San Raffaele Bio-medical Park Foundation, Rome, Italy and [3]Department of Histology and Medical Embryology, 'La Sapienza' University of Rome, Rome, Italy

During embryonic development and adult life, the plasticity and reversibility of modifications that affect the chromatin structure is important in the expression of genes involved in cell fate decisions and the maintenance of cell-differentiated state. Epigenetic changes in DNA and chromatin, which must occur to allow the accessibility of transcriptional factors at specific DNA-binding sites, are regarded as emerging major players for embryonic and hematopoietic stem cell (HSC) development and lineage differentiation. Epigenetic deregulation of gene expression, whether it be in conjunction with chromosomal alterations and gene mutations or not, is a newly recognized mechanism that leads to several diseases, including leukemia. The reversibility of epigenetic modifications makes DNA and chromatin changes attractive targets for therapeutic intervention. Here we review some of the epigenetic mechanisms that regulate gene expression in pluripotent embryonic and multipotent HSCs but may be deregulated in leukemia, and the clinical approaches designed to target the chromatin structure in leukemic cells.
Leukemia (2008) **22,** 1503–1518; doi:10.1038/leu.2008.141; published online 12 June 2008
Keywords: histone code; DNA methylation; leukemogenesis; epigenetic drugs

The hidden secrets of Pandora's box: chromatin and epigenetics

In-depth knowledge of chromatin architecture, as well as the epigenetic mechanisms that control it, are a solid source of hope in the effort being made to reestablish the correct pattern of gene expression in complex diseases such as cancer. In this article, we review the leading paradigms of DNA and chromatin epigenetic regulation, whose role may, we believe, be compared to that of 'hope' in the Greek myth of Pandora's box.

Chromatin is constituted by histone proteins and DNA. The central unit of chromatin is the nucleosome, which consists of about 146 bp of DNA wrapped around an octamer of core histone proteins: histone 2A (H2A), histone 2B (H2B), histone 3 (H3) and histone 4 (H4). The distance between nucleosomes, and thus their degree of compaction, defines two major chromatin structures: (1) euchromatin or 'active/open chromatin', which is characterized by a low degree of nucleosome compaction that permits the access of transcriptional machinery, which in turn allows transcription; (2) heterochromatin or 'inactive/closed chromatin', in which the high degree of nucleosome compaction impedes the access of transcriptional machinery and prevents transcription.

Nuclear chromatin architecture and its structure at specific loci are determined by heritable mechanisms that cause changes in gene expression without altering the DNA sequence, named epigenetics. These include DNA methylation, histone tail modifications, rearrangement of nucleosomal positioning and mechanisms of epigenetic targeting guided by noncoding RNAs.[1–6]

The equilibrium between the activities of different chromatin modifier enzymes determines heterochromatic and euchromatic chromatin states.[7]

DNA methylation

As regards DNA methylation, methylation of cytosine at cytosine-phosphate-guanine (CpG) dinucleotides within gene regulatory DNA sequences by DNA methyltransferases (DNMTs) influences the transcription of the related gene.[8–10] Table 1 lists the DNMT family members, their activities and relative inhibitors.[8,11–16] CpG dinucleotides are relatively uncommon and have an asymmetrical distribution throughout the eukaryotic genome. CpGs are gathered in repetitive sequences, above all around gene promoters in regions known as CpG islands.[17] In a normal cell, promoter CpG islands are generally unmethylated, whereas sparse CpGs tend to be predominantly methylated.[18] In most cases, methylated-promoter CpGs disable the transcription of the correlated gene.[19,20] However, it has become evident that the effects on transcription of CpG island-promoter methylation may depend on other, concomitant epigenetic events. These include the recruitment of repressive complexes containing methyl-CpG binding proteins, and the post-translational modifications of histone tails, which induce an inactive compacted chromatin status.[21]

Histone acetylation/deacetylation and histone methylation/demethylation

The histone tail modifications studied most, on account of their association with active/inactive chromatin states, are histone acetylation/deacetylation and histone methylation/demethylation. Acetylation of the H3 lysine 9 and 14 (H3K9acet and H3K14acet) correlates with accessible euchromatin and is associated with gene transcription. The level of lysine acetylation depends on the contrasting activities of the histone acetyltransferase (HAT) and histone acetyl-deacetylase (HDAC) groups of enzymes (Tables 2 and 3).[22–25] Conversely, histone lysine methylation depends on the contrasting activities of the

Correspondence: Professor C Nervi, Department of Histology and Medical Embryology, 'La Sapienza' University of Rome, Rome, Italy and San Raffaele Bio-medical Park Foundation, Via di Castel Romano 100, Rome 00128, Italy.
E-mail: clara.nervi@uniroma1.it
Received 12 October 2007; revised 24 April 2008; accepted 5 May 2008; published online 12 June 2008

histone methyltransferases (HMTs) and histone demethylases (HDMs) groups of enzymes (Tables 4 and 5).[26–29] Histone methylation correlates with both permissive and nonpermissive chromatin states, and consequently with either transcriptional activation or repression.[26] Histone H3 lysine 4 trimethylation (H3K4me3) marks permissive/open chromatin and gene activation, whereas H3 lysine 27 (H3K27me3) and 9 (H3K9me3) trimethylation mark nonpermissive/closed chromatin and gene inactivation. However, histone H3K27 and K4 lysines can be mono-, di- or trimethylated by polycomb/trithorax protein activity.[26–30,31]

The polycomb/trithorax protein complexes

The polycomb group of proteins (PcGs) are evolutionarily conserved transcriptional repressors, first identified in *Drosophila melanogaster* as repressors of homeotic genes (*Hoxs*). The trithorax (TrxGs) group of proteins acts antagonistically to PcGs to maintain gene transcriptional activation.[32]

The polycomb-repressor complexes (PRCs), PRC-1 and PRC-2, are implicated in the regulation of stem cell function.[33] The PRC-2 complex comprises three core components: the suppressor of zeste (Suz12), the embryonic ectoderm development (Eed1/3/4) and the HMT enhancer of zeste homologue 2 (Ezh2), which trimethylates the K27 and, to a lesser extent, the K9 on H3.[34] The trimethylation of H3K27 provides a binding platform for the recruitment of the repressive PRC-1 complex. The PRC-1 complex includes the mammalian methyl lysine cromodomain containing binding proteins (Cbx2/4/8), zinc-finger proteins (Edr1/2/3), ring-finger proteins (Ring1A/B, Bmi1, Pcgf2 and Znf134) and the sequence-specific DNA binding protein (Yin and Yang 1 (Yy1), sex comb on midleg-like 1 isoform (Scml1) and PHD finger protein 1 (Phf1)).[33] The recruitment of PRC-1 elements to appropriate genomic locations induces chromatin condensation and transcriptional gene silencing.[35,36] The exact mechanism of PRC-induced repression and the exact mechanism of PRC recruitment onto the DNA have yet to be fully clarified. Nonetheless, PRCs may block the formation of the transcription-initiation complex and inhibit gene transcription by: (1) abolishing ATP-dependent nucleosome remodeling by the Swi/Snf complex;[37,38] (2) inducing chromatin compaction by the methylation of H1K26, which binds the Hp1;[39] (3) recruiting DNMTs on selected genes;[40] (4) ubiquitylation of H2AK119;[36] (5) altering the topology of DNA through the formation of negative superhelical turns.[41]

By contrast, the Trithorax (TrxGs) family members sustain gene expression, including that of *Hox* genes, thereby allowing the formation of a permissive/open chromatin structure.[42] The mammalian TrxGs group includes several HMTs such as Set1A, Set1B and mixed-lineage leukemia (MLL) 1, 2, 3 and 4, which are responsible for the trimethylation of H3K4 (Table 4).[27] The *MLL* product is a multidomain molecule, which contains regions of homology with diverse proteins and is part of a multiprotein complex involving many components of the TFIID transcription complex. Close to the MLL N terminus there are: (1) three AT hooks, which probably stabilize protein–DNA interaction or mediate protein-–protein interactions by binding DNA; (2) two transcriptional repression domains. The first domain (RD1/repression domain 1) contains a DNMT1 homology domain including the CXXC zinc-finger domain, which recruits the polycomb-repressor proteins Hpc2 (human polycomb 2 homologue, also known as Cbx4), Bmi1 and the corepressor CtBP (C-terminal-binding protein).[43] The second domain (RD/repression domain 2) mediates transcriptional repression through the recruitment of HDACs and also interacts with a part of RD1.[43] It is noteworthy that MLL possesses HMT activity and recruits HATs, such as MOF and CBP (core-binding protein) on target genes.[44–46] Regardless of the interaction between MLL and multiple proteins that suppress gene expression (that is HDAC 1

Table 1 Examples of DNA methyltransferases, activity and relative inhibitors[8,11–16]

Enzyme	*Activity*	*Inhibitor*
DNA methyltransferase 1 (DNMT1)	Maintenance of DNA methylation pattern	5-azacytidine 5-aza-2′-deoxycytydine
DNA methyltransferase 3a (DNMT3a)	*de novo* DNA methylation	5-azacytidine 5-aza-2′-deoxycytydine
DNA methyltransferase 3b (DNMT3b)	*de novo* DNA methylation	5-azacytidine 5-aza-2′-deoxycytydine
DNA methyltransferase 3-like (DNMT3L)	It lacks canonical DNA methyltransferase motif it associates to DNMT3A, B	5-azacytidine 5-aza-2′-deoxycytydine

Table 2 Examples of histone acetyltransferases, post-translational modification, targets and relative inhibitors[22–23,25]

Histone acetyltransferase (HAT)	*Post-translational modification*	*Targeted histone and lysine*	*Inhibitor*
Human GNAT family members	Acetylation	H3K14/K9//K18	Butyrolactone (MB-3); MC1626
Gcn5	Acetylation	H3K14/K9//K18	Isothiazolones; H3-CoA-20
PCAF			
Human MYST family members	Acetylation	H4K5/K8/K12/K16	Anacardic acid
Tip60	Acetylation	H4K5/K8/K12	NA
HBO1	Acetylation	H4K16	Anacardic acid
MOZ	Acetylation	?H4K16	Anacardic acid
MORF	Acetylation		Anacardic acid
MOF			
p300/CBP family	Acetylation	H3K14/K18	Garcinol; anacardic acid; curcumin; Lys-CoA; isothiazolones; cynammoil derivatives 1a-c/2a-d
p300		H4K5	
CBP		H2AK5	
Usually referred to as a single entity because of their structural and functional homology		H2BK12/K15	

Table 3 Examples of histone deacetylases, post-translational modification, targets and relative inhibitors[24,25]

Histone deacetylase (HDAC)	*Post-translational modification*	*Targeted histone and lysine*	*Inhibitor*
Class I HDACs HDAC 1 HDAC 2 HDAC 3 HDAC 8	Deacetylation Deacetylation Deacetylation Deacetylation	No specificity reported	Short-chain fatty acids: sodium butyrate; phenyl butyrate; valproic acid; AN-9; *N*-hydroxy-4-(4-phenylbutyrylamino) benzamide (HTBP) Hydroxamic acids: trichostatin A; SAHA; LAQ824; LBH589; PXD101 cyclic peptides: depsipeptide Benzamides: CI994; MS275; MGCD010; trapoxin Cyclic tetrapeptide: apicidin
Class II2a HDACs HDAC4 HDAC5 HDAC7 HDAC9	Deacetylation Deacetylation Deacetylation Deacetylation	No specificity reported	Short-chain fatty acids: sodium butyrate; phenyl butyrate; valproic acid; AN-9; *N*-hydroxy-4-(4-phenylbutyrylamino) benzamide (HTBP) Hydroxamic acids: trichostatin A; SAHA; LAQ824; LBH589; PXD101 Benzamides: CI994; MS275; MGCD010; trapoxin Cyclic tetrapeptide: apicidin
Class II2b HDACs HDAC6 HDAC10	Deacetylation Deacetylation		
Class III HDACs Sirt1–3 Sirt4–7NAD+ dependents HDACs	Deacetylation	H4K16 (Sirt1–3)	Nicotinamide

Table 4 Examples of histone methyltransferases, post-translational modification, targets and relative inhibitors[26–28]

Histone methyltransferase (HMT)	*Post-translational modification*	*Targeted histone and lysine*	*Inhibitor*
ASH1L (*trithorax protein)*	Methylation	H3K4 to H3K4me3	
MLL1 (*trithorax protein)*	Methylation	H3K4 to H3K4me2	
MLL2 (*trithorax protein)*	Methylation	H3K4 to H3K4me3?	
MLL3 (*trithorax protein)*	Methylation	H3K4 to H3K4me3?	
MLL4 (*trithorax protein)*	Methylation	H3K4 to H3K4me?	
MLL5 (*trithorax protein)*	Methylation	H3K4 to H3K4me?	
SET1A	Methylation	H3K4 to H3K4me2/3	
SET1B	Methylation	H3K4 to H3K4me2/3	
EHMT1	Methylation	H3K9 to H3K9me2	
G9a	Methylation	H3K9 to H3K9me2	BIX-01294
PRDM2	Methylation	H3K9 to H3K9me3?	
SETDB1	Methylation	H3K9me1 to H3K9me3	
SETDB2	Methylation	H3K9me1 to H3K9me3	
SUV3-9H1	Methylation	H3K9me1 to H3K9me3	
SUV3-9H2	Methylation		
EZH2 (polycomb protein)	Methylation	H3K27 to H3K27me3	3-Deazaneplanocin A (DZNep)
NSD1	Methylation	H3K36 to H3K36me2	
SET2	Methylation	H3K36 to H3K36me2/me3?	
DOT1	Methylation	H3K79 to H3K79me3	

and 2, PcG proteins Hpc2 and CtBP), genetic and biochemical evidence points to MLL as a positive regulator of gene expression for known targets including *Hox* genes.[44,47] MLL methylates H3K4, thereby providing a gene activation mark on the targeted chromatin that regulates *Hox* gene expression during the development of hematopoietic stem cells (HSCs).[47] The regulation of *Hox* genes by wild-type MLL involves both the SET methyltransferase domain, which mediates histone methylation of H3K4, and the recruitment of HATs, such as MOF and CBP.[44] Furthermore, the recent development of ChIP-on-chip technology has yielded data revealing that MLL is associated with thousands of promoters, the vast majority of which are occupied by RNA polymerase II, which thus suggests that MLL is important in the regulation of transcription.[47,48]

The correct activity of the transcriptional machinery may, therefore, depend on the accessibility of target DNA sequences to specific promoters, enhancers and insulators. The accessibility of target DNA sequences depends on the chromatin status, which affects the establishment of stable binding between transcription factors and their cognate sequences. Thus, we hypothesize that chromatin serves as a sort of GPS (global positioning system), insofar as it guides the appropriate transcription factors toward specific routes for development and differentiation. As discussed below, chromatin-based information may explain why tissue-specific genes required for executing terminal differentiation programs are not expressed in either pluripotent embryonic stem (ES) cells or multipotent HSCs, even though their expression potential is retained.

Table 5 Examples of histone demethylases , post-translational modification, targets and relative inhibitors[29]

Histone demethylase (HDT)	*Post-translational modification*	*Targeted histone and lysine*	*Inhibitor*
LSD1	Demethylation	H3K4me2 to H3K4 H3K4me1 toH3K4 H3K9me2 to H3K9	*trans*-2-Phenylcyclopropylamine (2PCPA)
JmjC family:	Demethylation	H3K36me2 to H3K36	
JHDM1a/FBXL11	Demethylation	H3K36me1 to H3K36	
JHDM1b	Demethylation	H3K9me2 to H3K9	
JHDM2/JMJD1A		H3K9me3 to H3K9me2	
JHDM3		H3K36me3 to H3K36me2	
JMJD2 family: JMJD2A	Demethylation	H3K9me3 to H3K9me2	
JMJD2B	Demethylation	H3K36me3 to H3K36me2	
JMJD2C	Demethylation	H3K9me3 to H3K9me2	
JMJD2D	Demethylation	H3K36me3 to H3K36me2	
JMJD3/UTX	Demethylation	H3K9me3 to H3K9me2 H3K9me3 to H3K9me2 H3K9me2 to H3K9 H3K27me3to H3K27	
JARID1 family (A–D)	Demethylation	H3K4me3 to H3K4	

Self-renewal and lineage specification of embryonic and hematopoietic stem cells: role of transcription factors, chromatin architectures and modifications

Stem cells are characterized by their capacity to both self-renew and produce differentiated functional cell types. However, whereas pluripotent ES cells derived from the embryo can self-renew and generate all the body cell types in culture and *in vivo*, multipotent cells such as HSCs can self-renew and give rise to all the cell lines only in a particular lineage.

Embryonic stem cells

The differentiation of ES cells from totipotent to pluripotent and, consequently, to developmentally more restricted states, is endorsed by changes in the expression of transcription factors related to chromatin remodeling and modifications.[49] In this regard, an important breakthrough was recently made by Takahashi and Yamanaka[50] who, by means of the viral-mediated transduction of four transcription factors, that is Oct4, Sox2, c-Myc and Klf4, successfully reprogrammed mouse embryonic/adult fibroblasts and different human somatic cells to pluripotent ES-like stem cells (namely iPS cells).[50,51] It has been suggested that the ectopic expression of these reprogramming factors in infected somatic cells initiates a sequence of epigenetic events, including changes in DNA methylation and chromatin modifications in endogenous genes that are important in the maintenance of ES pluripotency and lineage specification (that is *Oct4*, *Sox2* and *Nanog*), thereby triggering the pluripotent state of iPS cells.[49,50,52]

Indeed, unique, plastic epigenetic marks characterize the maintenance of the capacity of self-renewal, pluripotency and the activation of cell lineage specifications in ES cells. Undifferentiated ES cells display a more open chromatin state at the genomic wide level and a higher exchange rate of chromatin-associated proteins than differentiated cells.[53–55] Moreover, the differentiation of human and mouse ES cells is accompanied by a general change in nuclear architecture and by changes in chromatin structure, above all at loci involved in maintaining pluripotency and in inducing lineage-restricted programs of gene expression.[56]

These findings are supported by other authors who have reported changes in heterochromatin marks (H3K9me3 and H3K27me3) and euchromatin marks (acetylated forms of histones H3, H4 and H3K4me3) during ES cell-induced differentiation.[53,57] For example, *Oct4* and *Nanog* gene expression, which is required to maintain the pluripotency of ES cells, requires active chromatin marks such as the acetylation of H3 and H4 on their promoter regions. Notably, experimental evidence from two independent laboratories has shown that ES cells retain specific histone modifications in the promoters of lineage-control genes not expressed in ES cells, which belong to the *Sox*, *Hox*, *Pax* and *Pou* gene families, and include *Sox1*, *Nkx2-2*, *Msx1*, *Irx3* and *Pax3* genes.[58,59] Many of the regulatory regions in these genes are, unexpectedly, marked by histone modifications that both activate (H3K9acet and H3K4me3) and repress (H3K27me3) gene transcription. This bivalent nature of histone modifications has been proposed as the mechanism through which ES cells: (1) retain their pluripotency; (2) impede the expression of lineage-specific genes (due to the dominant effect of the repressive mark H3K27me3); (3) prime lineage-specific genes for activation or inhibition in subsequent phases of terminal differentiation (Figures 1a–c). Thus, the transcription of lineage-specific genes may be poised by dynamic patterns of histone marks, which are differentially interpreted by cellular transcription factors according to the gene locus and cellular context, as opposed to static histone marks that merely switch gene transcription on and off.[25,60] In ES cells, however, other lineage control genes are not marked by any known or detectable histone modifications, or are only marked by an activating H3K4me3 mark.[59]

DNA methylation also affects ES cell chromatin structure and the appropriate activation of differentiation programs. The relevance of proper DNA methylation patterns becomes evident when ES cells are depleted of DNMTs. The consequent loss of DNA methylation results in increased cellular apoptosis in embryos and in impaired differentiation of undifferentiated ES cells.[61,62] It is noteworthy that appropriate DNA methylation patterns are essential to improve animal cloning efficiency when somatic cell nuclei are used in nuclear transfer experiments.[63]

Replication timing program is another important cellular tool for the establishment of a correct transcriptional profile in a particular cell type.[64] The replication timing of specific genes

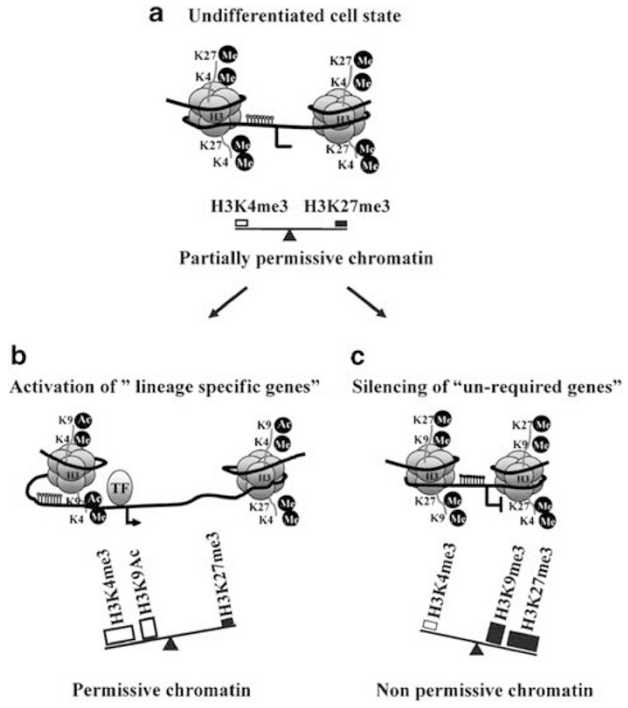

Figure 1 Chromatin status and histone tail modifications in gene regions regulating embryonic stem (ES) cells pluripotency and lineage specification. (**a**) Undifferentiated cell status: a balanced bivalent nature of nucleosomal histone modifications including H3 lysine 4 trimethylation (H3K4me3) activating mark and H3 lysine 27 trimethylation (H3K27me3) repressive mark define the 'partially permissive chromatin' status in specific gene regulatory regions. This unusual bivalent pattern is modified when ES cells are induced into lineage-specific differentiation programs. (**b**) Activation of lineage differentiation programs: regulatory regions of specific genes acquire a 'permissive chromatin status', which is characterized by high levels of activating histone marks, such as H3K4me3 and H3K9acet and unmethylated cytosine-phosphate-guanines (CpGs), and by low levels of repressive histone marks, such as H3K4me3 and methylated CpGs. (**c**) Silencing of genes not required for lineage specification: gene promoters become unavailable to transcription factors through the formation of a 'nonpermissive chromatin' status characterized by methylated CpGs, high levels of the repressive histone marks H3K27me3 and H3 lysine 9 trimethylation (H3K9me3), and lack of the activating marks H3K4me3 and H3K9acet. Gray spheres indicate the octamer of histones forming the nucleosome with the double strand DNA wrapped around them. At the bottom of each panel, the beam balance shows the 'weight' of the different histone tail modifications in rendering chromatin partially permissive, permissive and nonpermissive for transcription. Histone-activating marks (white box); histone-repressive marks (black box); H3, histone 3; K, lysine; me, methylation; me3, trimethylation; ac, acetylation; unmethylated CpGs (white circles); methylated CpGs (black circles); TF, transcription factor.

depends on chromatin structure. In ES cells it may be essential for maintaining their pluripotent status or for executing terminal differentiation programs. Replication timing reflects chromatin changes and depends on them, and is associated above all with increased histone acetylation. Azaura *et al*.[58] have shown, for example, that genes encoding neural-specific transcription factors replicate early in mouse ES cells but later in hematopoietic-restricted cells, in which the neural potential is lost. These data support the notion that epigenetic changes affecting the expression of genes that are essential for maintaining the pluripotent status and lineage-restricted programs may be required for switching to late or early replication as the differentiation program proceeds.[64–66]

Chromatin remodeling by polycomb/trithorax proteins. In keeping with findings indicating that the methylation status of H3K27 and H3K4 discerns the chromatin structure of lineage-specific genes in pluripotent/multipotent stem cells, whereas high H3K9acet or H3K9me3 levels are chromatin marks of differentiated cells,[67] it has been reported that H3K27 trimethylation is related mainly to the formation of 'optional' heterochromatin,[68,69] whereas H3K9 trimethylation is related mainly to 'stable' heterochromatin for gene silencing.[67,70,71]

Two independent studies on human and mouse ES cells have shown that PcG proteins bind to and then repress the promoters of homeodomain-containing transcription factors, such as *Dlx*, *Irx*, *Lhx*, and *Pax*, which regulate neurogenesis and hematopoiesis.[72,73] Furthermore, PcG proteins suppress the activity of the *Fox*, *Sox*, *Gata* and *Tbx* transcription factor family members and of signaling molecules such as *Wnts*, *Shh* and *Bmps*, which is important in the development and disease.[74] These data point to a model in which PcG proteins in ES cells or in multipotent progenitors crucially silence genes involved in development and cell differentiation, thereby retaining a pluripotent/multipotent cell population. On the basis of the data available, it is thus possible to hypothesize that lineage control genes in these cells are bound by PcGs and TrxGs, and are maintained in a 'relatively permissive' chromatin conformation by the balance between the active histone mark H3K4me3 and the inactive histone mark H3K27me3 (schematically shown in Figure 1a). This relatively permissive chromatin conformation would make lineage control genes accessible for both chromatin-remodeling complexes and transcription factors for subsequent transcriptional activation or inhibition in response to appropriate microenvironmental signals (Figure 1b). As the differentiation program proceeds, the chromatin of genomic loci involved in the differentiation program would acquire better defined, more stable characteristics.

Although more studies are needed to establish the causal interplay between changes in chromatin status, gene expression, maintenance of pluripotency and lineage-restricted differentiation, we should consider epigenetic modifications essential for the maintenance of pluri- and multipotency and for the correct onset of differentiation programs in both ES and HSCs.

Hematopoiesis

The model proposed for ES cells easily fits into both the concept of hierarchical gene activation and the importance of hematopoietic transcription factors in inducing chromatin remodeling at different stages of hematopoiesis, the life-long, highly regulated multistage process through which a multipotent self-renewing HSC gives rise to all blood cell lineages.[75] During embryogenesis and postnatal life, the development, self-renewal capacity, lineage commitment and maturation of HCS into erythroid, granulocytic, monocytic and megakaryocytic lineages are dictated by two closely related events: (1) the composition of external signals from the bone marrow microenvironment, including soluble growth factors, cell–cell and cell–extracellular matrix interactions; (2) the sequential, coordinated and combinatorial expression/activity of intrinsic lineage-affiliated transcription factors, which bind regulatory DNA sequences, modulate specific gene expression programs and act as master regulators.[76,77]

For instance, transcription factors Scl/Tal1 (T-cell acute lymphocytic leukemia-1 protein) and AML1 (also named Runx1 from runt-related transcription factor 1 or CBFA2) are potent regulators of HSCs. Their depletion affects the entire blood cell differentiation process. By contrast, transcription factors such as

Gata1 (GATA-binding protein 1), C/ebpα (CCAAT enhancer-binding protein α) and Pu.1 have a more restricted expression pattern, their activity being related to lineage-specific determination. A number of mechanisms have been proposed to explain how these transcription factors determine the onset and maintenance of lineage differentiation; the mechanisms proposed have either an antagonistic or cooperative effect on gene expression patterns.[77,78] As hypothesized for Oct4 and Nanog in ES cells, the expression of some hematopoietic transcription factors may be dependent on specific chromatin modifications. In addition, transcription factors may either recruit or, as multiprotein complexes that perform chromatin modifier activities, prime the chromatin for the stable binding of other factors and for long-term lineage-specific gene activation/repression.[79]

For example Gata1, a sequence-specific transcriptional activator expressed in megakaryocytic and erythroid cells, is important in the control of lineage-specific programs related to erythroid differentiation. During human erythropoiesis, the maturation of erythrocytes is associated with the increased expression of α- and *β-globin* genes, which are required for synthesizing hemoglobin. Gata1 acts by stably interacting with chromatin in regulatory regions on target genes, including α- and *β-globin* genes. At these sites, Gata1 induces a transcriptionally permissive chromatin configuration by recruiting protein complexes containing HAT activity (CBP).[80,81] Interestingly, in nonerythroid cells, *globin* genes exist in a methylated, transcriptionally silent state.[82,83] However, *Gata1* promoter is itself a target of epigenetic modifications that regulate its own expression during erythroid differentiation[83] (schematically represented in Figure 2a). In HSCs and in multipotent progenitors (MPPs), *Gata1* expression is very low and its promoter chromatin is marked by low levels of active and inactive chromatin marks (H3K4me3, H3K9acet and H3K27me3). As differentiation proceeds to common myeloid precursors (CMPs) and megakaryocyte/erythrocyte precursors (MEPs), H3K4me3 accumulates along the Gata1 promoter region in both CMP and MEP, thereby allowing the formation of a permissive chromatin status and the expression of *Gata1*. By contrast, high levels of the inactive chromatin mark H3K27me3 are found on the *Gata1* promoter and correspond to the silencing of *Gata1* in common lymphoid (CLP) and granulocyte/monocyte (GMP) progenitors[83] (Figure 2a).

Moreover, Gata1 physically interacts with Pu.1 when Pu.1 is in a complex with pRb. The binding of Pu.1 to Gata1 sites on *Gata1* target genes leads to the inhibition of the erythroid lineage and activation of the myeloid differentiation program. This is the consequence of the recruitment, at these sites, of the H3K9 methyltransferase Suv39H1 and Hp1 protein (heterochromatin protein 1) by Pu.1, which confers a 'nonpermissive' characteristic to the chromatin.[84] It is noteworthy that the silencing of Pu.1 releases this repressive complex, erases the repressive chromatin marks and reactivates the erythroid program.[84]

Although *Gata1* promoter does not contain a proper CpG island, the eight CpGs located upstream of the transcriptional start site are progressively demethylated and the *Gata1* expression level increases as the differentiation of HSC proceeds toward CMP and MEP.[83] It is noteworthy that the same CpGs maintain a methylated status in CLP and GMP.[83]

Scl/Tal1 is expressed in HSC and acts as a positive regulator of erythroid differentiation. Whereas the acetylation of Scl/Tal1 drives murine erythroleukemia cells toward erythroid differentiation, its association with the transcriptional repressor complex mSin3A/HDAC blocks cell maturation.[85]

Other hematopoietic cell lineage programs also require controlled expression of specific transcription factors. For instance, the transcriptional activator Pu.1 is required for the development along the lymphoid and myeloid lineages but needs to be downregulated during erythropoiesis.[86] Moreover, *Pu.1* is expressed in HSCs and in differentiated B cells, though not in T cells (CD4+ and CD8+), where its regulatory 5′ region is hypermethylated.[87,88] *Pu.1* gene expression is upregulated during myeloid differentiation and enables committed

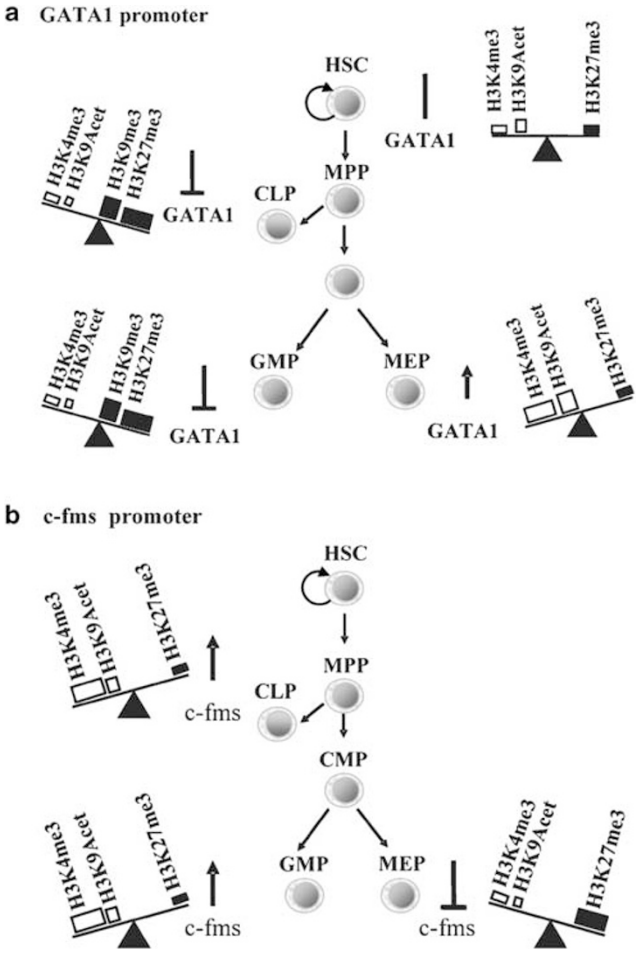

Figure 2 Epigenetic marks characterizing *Gata1* and *c-fms* regulatory regions during erythropoiesis and granulocytopoiesis. (**a**) *GATA1* is a target of epigenetic modifications that regulate its expression during erythroid differentiation. In multipotent hematopoietic stem cells (HSCs), *Gata1* expression is almost undetectable and the chromatin in its regulatory region is marked by low levels of activating (H3 lysine 4 trimethylation, H3K4me3 and H3K9acet) and repressive histone marks (H3 lysine 27 trimethylation, H3K27me3 and H3 lysine 9 trimethylation, H3K9me3). As the differentiation of hematopoietic stem cells (HSCs) proceeds to megakaryocyte/erythrocyte progenitors (MEP), the activating marks (H3K4me3 and H3K9acet) accumulate along the *Gata1* promoter region, thereby allowing the formation of a permissive chromatin status and the expression of *Gata1*. By contrast, a nonpermissive chromatin status is enforced in common lymphoid progenitors (CLPs) and granulocyte/macrophage progenitors (GMPs) where *Gata1* expression is not required. (**b**) *c-fms* regulatory elements, except for the FIRE region, are enriched with the activating histone mark H3K4me3 in CLP, common myeloid progenitors (CMPs) and GMP. By contrast, high levels of the repressive histone mark H3K27me3 are detected in MEP, where they mark the permanent silencing of c-fms. The beam balance shows the 'weight' of the various histone tail modifications (defined in the legend of Figure 1), that at different stages of hematopoiesis influences the chromatin status of *GATA1* or *c-fms* promoters and in relation to their induced (↑) or silenced (⊥) transcription.

macrophage precursors to respond to colony-stimulating factor 1. Indeed, albeit with other transcription factors, Pu.1 regulates the expression of *c-fms* (macrophage colony-stimulating factor receptor), which is crucial for the growth and differentiation of the monocyte–macrophage lineage.[89] The expression of *c-fms* is highly regulated by a control region that includes a promoter sequence, spans the transcriptional start site and the c-fms intron regulatory element termed FIRE, and performs macrophage-specific enhancer activity.[90] Krysinska *et al*.[91] have shown that in *Pu.1*−/− cells, *c-fms* chromatin is accessible to the binding of transcription factors, even though it lacks inactive (H3K9me3) and active (H3K9acet and H3K4me3) histone marks. Following *Pu.1* induction, *c-fms* promoter is bound by Pu.1; nonetheless, *c-fms* mRNA expression level remains low and activating histone marks are not observed on *c-fms* regulatory regions. The appearance of activation marks (H3K9acet and H3K4me3) along the *c-fms* promoter and the increase in the *c-fms* mRNA transcription follow the Pu.1-dependent expression of the Egr2 (early growth response 2 protein), which causes the reorganization of chromatin at the *c-fms* FIRE region. Indeed, Egr-2 binding to the FIRE region of *c-fms* gene is required for the hierarchical binding of other regulators, such as Pu.1 itself and C/ebpβ, and for the recruitment of the acetylating enzyme CBP and Brg1, a component of the ATP-dependent chromatin-remodeling complex Swi/Snf. Attema *et al*.[83] have also reported that epigenetic modifications in the *c-fms* promoter and FIRE regions occur simultaneously with changes in *c-fms* mRNA levels during hematopoiesis. Except for the FIRE region, regulatory elements on the c-fms 5′ region are enriched with the active chromatin mark H3K4me3 in CLP, CMP and GMP. In GMP, *c-fms* expression is required to allow the committed macrophage precursors to respond to colony-stimulating factor 1. By contrast, high levels of the inactive chromatin mark H3K27me3, which reveal the permanent silencing of c-fms, are detectable at this gene site in MEP (Figure 2b).

Moreover, it is possible, on the basis of the CpG methylation level, to identify three DNA methylation patterns in the *c-fms* transcriptional regulatory region:[83] (1) CpGs within the promoter and the FIRE regions, most of which are unmethylated in hematopoietic populations; (2) the lowest CPG methylation levels within the promoter are observed in GMPs that express *c-fms*; (3) CpGs in the 1 kb region downstream of the FIRE site, most of which are methylated in hematopoietic populations, partially methylated in CLP and fully methylated in liver, where c-fms activity is not required. The lowest methylation densities in this region are detected in the CMP and GMP subpopulations, with slightly higher levels in MEP.

Another example of chromatin priming by hematopoietic transcription factors is provided by *Mim-1* expression, which accompanies myelomonocytic differentiation.[92] *Mim-1* is not expressed in HD50 multipotent progenitor cells, but is highly expressed in the HD50myl cell line that is committed to the myelomonocytic lineage. Plachetka *et al*.[92] studied the chicken *Mim-1* gene coding for the myeloid protein 1, showing that its expression depends on both C/ebpβ and c-Myb binding. In particular, the recruitment of p300/CBP acetylating activity by C/ebpβ in the *Mim-1* gene results in chromatin reorganization of *Mim-1* enhancer. This is not, however, sufficient to activate *Mim-1* transcription, which requires *Myb* binding in addition to *C/ebpβ*-dependent chromatin reorganization in the promoter region.

The expression of *Gata3* and *Ptcrα* (T-cell antigen receptor α) is associated with lymphoid lineages. *Gata3* and *Ptcrα*-promoter regions are enriched with active chromatin marks (H3K4trimet and H3acet) in MPP and CLP. However, increased levels of the inactive chromatin mark H3K27me3 are measurable in the *Gata3* and *Ptcrα*-promoter regions, in most of the erythro-myeloid populations and, unexpectedly, in CLP. This reveals the existence of a dual, opposite mark in the same regulatory region whose function cannot easily be interpreted at this stage of differentiation.[83]

All these data support the notion that transcription factors may have additional roles besides the mere activation and inhibition of transcription. These include their ability to trigger the initial steps of chromatin opening in the enhancer and promoter regions of lineage-specific genes, which determines the switch from partially permissive chromatin to fully permissive or nonpermissive chromatin, thereby allowing the stable interaction of transcriptional machinery and the formation of lineage-specific chromatin structures.

The leukemogenic potential of altered DNA methylation status, aberrant chromatin-remodeling and/or polycomb/thritorax activities

Genes encoding hematopoietic transcription factors, including *C/ebpα*, *AML1*, *Gata1*, *Pu.1* and *MLL*, can be mutated or altered by chromosomal translocations in leukemias. This suggests that the dysregulation of transcription factor activity is important in the pathogenesis of the differentiation block characterizing these malignancies.[78,93] However, epigenetic alterations may also participate in the earliest stages of neoplasia by affecting the transcription of genes regulating stem/precursor cell development and lineage specification.[94,95] This may, *per se* or in association with genetic alterations, lead to clonal expansion, the block of hematopoietic precursor differentiation and leukemogenesis. For example, in leukemias in an anomalous background of genome-wide hypomethylation associated with increased genomic instability, a high number of genes are hypermethylated and not expressed. The products of these genes belong to different functional classes including: cell-cycle regulators (p16INK4a, p15INK4b and p21WAF), proapoptotic proteins (Dapk1 and Crbp1), DNA repair enzymes (Mgmt), signal transduction molecules (Socs1), metastasis/invasion (Cdh1), transcriptional regulators (C/ebp and Meis1), nuclear receptors (ER and RARβ2), metabolism (Gstp1) and genome stability (Lats2).[95]

However, the mechanisms promoting aberrant DNA methylation and changes in chromatin patterns may be different. Specific fusion genes and fusion products may be associated with the differentiation block that characterizes distinct acute myeloid leukemia (AML) subtypes as classified by FAB, which relies on the morphologic and cytochemical characteristics of blasts[96] (examples are provided in Figure 3). In the cases documented best, such as AMLs presenting the *t*(8;21) and *t*(15;17) genetic translocation fusion products, leukemogenesis may be caused by an aberrant recruitment of protein complexes containing HDAC, HMT and DNMT activities, which alters chromatin structures and silences key myeloid genes.[97–100]

Rearrangements affecting the retinoic acid (RA) receptor α (*RARα*) gene on chromosome 17 are almost exclusively associated with the differentiation block that occurs in the promyelocytic stages of myelopoiesis. Indeed, in more than 90% of cases of acute promyelocytic leukemia (APL), the fusion oncoprotein PML/RARα is generated as a consequence of the *t*(15;17), which fuses the *PML* gene on chromosome 15 to that of the all *trans*-RARα present on chromosome 17. RARα is a member of the nuclear receptor superfamily and acts as a ligand-inducible transcription factor. Heterodimerization, with

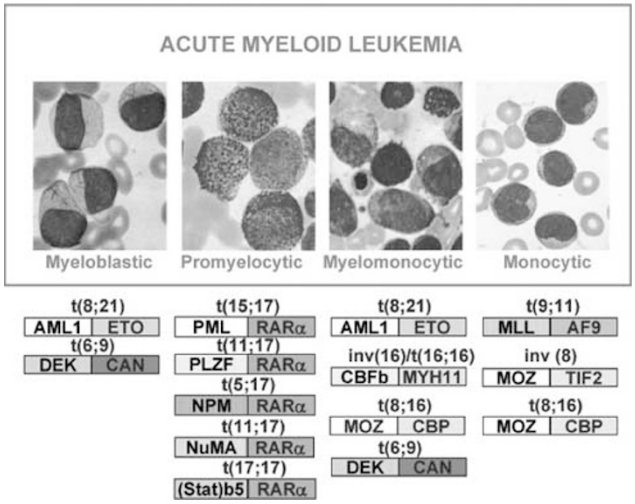

Figure 3 Examples of fusion genes and fusion products associated with a specific differentiation block in different acute myeloid leukemia (AML) subtypes. The AML1–ETO and CBFβ–MYH11 fusions, both of which lead to alterations in the CBFβ transcription complex, might disrupt hematopoiesis through distinct mechanisms, as they can be associated with myeloblastic and myelomonocytic AML subtypes (AML-M2 and AML-M4 by FAB classification).[96] Rearrangements affecting the retinoic acid (RA) receptor α (*RARα*) gene on chromosome 17 are exclusively associated with a differentiation block in the promyelocytic stages of myelopoiesis (AML-M3 by FAB classification). The MLL–AF9 oncogene originates from the translocation *t*(9;11)(p22;q23), which is associated above all with monocytic acute myeloid leukemia (AML-M5 by FAB classification). Less frequent translocations, such as DEK-CAN, give rise to fusion genes associated with myelodysplastic syndromes progressing to AML. Furthermore, transcriptional coregulators with putative histone acetyltransferase (HAT) activities (such as CBP, MOZ and TIF2), or adapter proteins that recruit the corepressor–histone acetyl-deacetylase (HDAC) complex (such as ETO), are present in chromosomal rearrangements associated with AML-M4/M5 by FAB classification.

its transcriptional coactivator retinoid X receptor (RXR) and its presence in protein complexes including HDAC and HAT activities, is essential for the effective ligand-dependent transactivation of specific RA target genes by RARα.[101,102] However, the oligomerization capacity of PML increases the affinity for transcriptional corepressors of the RARα moiety present in the PML/RARα product. This enhances the recruitment of chromatin modifiers (HDACs, HMTs, DNMTs and methyl-binding domain proteins (MBDs)) on *RA* target gene promoters, causing their transcriptional silencing.[103–106] Moreover, PML–RARα can also bind and recruit at its transcriptional target gene *RARβ2*, the polycomb-repressive complex-PRC2.[107] Interestingly, the knockdown of *Suz12*, a PRC-2 component, reverses histone marks and DNA methylation status at specific sites on the *RARβ2* gene promoter, resulting in its reactivation and the granulocytic differentiation of APL blasts.[107] However, two recent studies have shown that RXR, the heterodimeric partner of RARα, is a critical determinant for the transforming potential and maintenance of the leukemic phenotype of PML/RARα and other APL-associated RARα fusion products.[108,109] RXR may be required for the full execution of the transcriptional program imposed by PML/RARα on its downstream targets. This appears essential for leukemic transformation due to the enhanced DNA binding and gene target expansion of the PML/RAR–RXR heterooligomeric complex.[108,109]

The gene encoding AML1 is targeted by the *t*(8;21), the most frequent chromosomal rearrangement in AML (about 40% of the cases), which generates the AML1/ETO fusion product. AML1/ETO is constituted by a portion of the AML1 transcription factor fused to the corepressor ETO protein.[110–112] The AML1 moiety of the fusion product retains its DNA binding activity, whereas the ETO moiety conveys new properties to the fusion protein, including: (1) several docking sites for the corepressors SMRT, N-CoR and Sin3A and the histone deacetylases HDAC1, 2 and 3; (2) a dimerization domain that initiates the formation of homo-oligomers of the fusion protein, which increase its effects on target genes and associated proteins.[113] Therefore, thanks to these acquired activities, AML1/ETO can function as a transcriptional repressor of AML1-regulated genes by directly binding AML1 consensus sequences in their regulatory regions. However, the AML1/ETO oncoprotein also exerts its oncogenic activity through other mechanisms. For instance, AML1/ETO can physically interact with transcription factors, altering their proper activity and expression of their target genes. These AML1/ETO-induced aberrant mechanisms involve key regulators of hematopoiesis such as C/ebpα, Pu.1, the retinoid receptor RARα–RXR heterodimer, Gata1, a subset of E-box binding proteins including E2A, Heb and E2-2 and myelopoiesis regulator microRNA-223.[105,114–119] In addition, experimental evidence has shown that AML1/ETO expression upregulates genes such as *Jagged 1*, thus affecting the Notch pathway, *plakoglobin* and *β-catenin*, therefore increasing the activity of Wnt signaling system,[120,121] which may be relevant to the increase in the self-renewal potential and block of committed myeloid progenitor differentiation following the expression of AML1/ETO in hematopoietic stem/precursors.

Genes encoding transcriptional coactivators with putative HAT activities can also be targeted by genetic translocations associated with AMLs (Figure 3). The translocations *t*(8;16), *t*(10;16) and the inversion inv(8) fuse the human monocytic leukemia zinc-finger protein Moz (MYST3) and its paralog Morf (MYST4) acetyltransferases to genes encoding the nuclear receptor coactivators CBP and p300 or the p160 protein TIF2 (transcription intermediary factor 2). The resulting fusion proteins can transform hematopoietic progenitors *in vitro*, and induce myeloproliferative disease in mice. Recent findings indicate that Moz fusion proteins interfere with the activities of cellular CBP, nuclear receptors, p53 and AML1 proteins by promoting aberrant patterns of histone and nonhistone protein acetylation with a leukemogenic potential.[122–125]

The loss of Suv39H1-H3K9 methyltransferase generates genomic instability and a decrease in H3K9me3 levels, thereby favoring the onset of B and T-cell lymphomas in mice.[126,127] It has recently been suggested that other HMT alterations that target different histone lysines, such as H3K36 and H4K20 by Nsd1 and H3K79 by hDot1L, may be important in leukemogenesis.[128]

The function and timing of the alterations in polycomb and trithorax protein activities suggest that these activities are the most likely causes of the onset and progression of tumors, including leukemia, which occur through several mechanisms. TrxGs may promote the abnormal activation of oncogenes, although PcGs may induce transcriptional silencing of tumor-suppressor genes and regulate stem cell plasticity by repressing lineage-specific genes.[30,72,73] Thus, anomalous PcG activity might affect the correct expression of lineage-specific genes, thereby impeding the maturation of stem cells and committed progenitors, and allowing the onset and expansion of a 'cancer stem cell'. Polycomb proteins are, indeed, overexpressed in different tumor types. The close connection between *PcG* gene expression regulation, cancer and stem cell origin of cancer is supported by the independent, simultaneous work conducted by

three groups, who have shown that PRC-2 components, including H3K27 trimethylation, mark ES cells of genes that are frequently hypermethylated in cancer.[67,129,130] Interestingly, the polycomb protein Suz12, a component of the PRC-2 repressive complex, is overexpressed in colon, liver and breast cancers.[131] The H3K27 methyltransferase Ezh2, which also belongs to the repressive PRC-2 complex, is overexpressed in lymphomas, prostate, bladder and breast tumors, and is closely correlated with disease aggressiveness.[132] Downregulation of the PRC-2 complex protein Eed is associated with an increased incidence of carcinogen-induced lymphoma.[133] The polycomb ring-finger oncogene *Bmi1* is overexpressed in lymphomas, leukemia, neuroblastoma and non-small-cell lung cancer.[132] Its potential use as a prognostic marker in AML and in chronic myeloid leukemia has recently been proposed.[134,135] *Bmi1*, a known regulator of normal ES cell self-renewal, also sustains the self-renewal capacity of leukemic stem cells, as demonstrated by the reduced proliferation of leukemic cells lacking *Bmi1* and the consequent failure of leukemia transplantation in a mouse model of AML.[136,137] Moreover, *Bmi1* appears to exert its oncogenic activity by repressing p16 and c-Myc-induced apoptosis.[138,139]

Among mammalian TrxG proteins, *MLL* gene dysfunction is related to leukemogenesis. *MLL* rearrangements are present in more than 70% of infant leukemias, although *MLL* translocations are present in approximately 10% of AMLs in adults and in therapy-related leukemias.[140] *MLL* rearrangements erase the sequences conserved best, such as the central zinc-finger domain and the C-terminal SET domain. Various chromosomal abnormalities (translocations, inversions and interstitial duplications) involving *MLL* are clustered in a major break region just after the repression domain and are present in patients with myeloid or lymphoid leukemias.[141] The N-terminal part of the *MLL* gene fuses to the C-terminal part of a remarkable number (at least 36) of diverse partner genes. The most common translocation partners, that is *Enl*, *AF9*, *AF10* and *AF4*, belong to the family of serine/proline-rich nuclear proteins (for example MLL–ENL, MLL–AF9, MLL–AF4, MLL–AF10 and MLL–AF6) and function as transcriptional activators.[142] A second class of translocation partners of cytoplasmic origin (AF1P/Eps15, EEN, AFX, GAS7 and septins) dimerize with the truncated MLL form.[142] A third group includes HATs (p300 and CBP).[143–145] All fusion protein groups lack both the SET domain and the CBP and MOF interaction domain.

MLL gene also undergoes partial tandem duplications spanning the exons 5/11 and 5/12 (MLL-PTD).[142] However, in this case the SET domain is retained, which raises the question of whether perturbed H3K4 methylation is the only mechanism underlying MLL functional alterations.[146] For instance, the oligomerization properties of fusion partners (MLL–GAS7 and MLL–AF1P), or their direct role in transcriptional regulation (MLL–AF9 and MLL–ENL), may be equally relevant in conferring leukemogenic activity to MLL fusion products.[147–151] However, there is no doubt that some MLL fusion partners, besides the well-known CBP and MOF, belong to a network involved in transcriptional regulation through chromatin remodeling.[152] For example, the MLL fusion partner AF10 interacts with the HMT hDot1L, which methylates H3K79.[128] This gives the fusion product the ability to immortalize the hematopoietic progenitors, whereas in the absence of hDOT1L, MLL–AF10 is unable to transform the hematopoietic progenitors. hDot1L protein has also been found to bind AF9 and AF4.[153,154]

MLL rearrangements are also associated with the anomalous overexpression of the *Hox* genes and aggressive leukemias. Indeed, *MLL*-knockout mice display severe hematopoietic defects associated with defects in *Hox* gene (including *HoxA9*) expression, although the overexpression of selected *Hox* genes, such as *HoxA9* and the Hox cofactor *Meis1*, is implicated in human myelodysplastic disorders, in acute myeloid and lymphoid leukemias.[44,142,155] HoxA9 and Meis1 are normally expressed only in early hematopoietic lineages, their expression being downregulated to undetectable levels in later stages of differentiation. MLL fusion proteins enforce the persistent expression of HoxA9 and Meis1, which appears to be critical for leukemogenesis. Nonetheless, overexpression of HoxA9 induces stem cell expansion and is associated with poor-prognosis AML. However, when coexpressed with Meis1, HoxA9 is acutely transformed.[156,157] *HoxA9* and *Meis1* overexpression, which follow MLL–ENL induction, are also associated with increased H3K79 methylation and lack of H3K4me3.

Therapeutic potential of epigenetic cell reprogramming

ES cell reprogramming

Owing to their potential to generate all cell types in culture, ES cells have raised interesting new prospects regarding their therapeutic application in a wide variety of diseases, ranging from genetic or degenerative disorders to neoplasia. However, ES-based treatment could be complicated by difficulties regarding immune rejection due to the immunological incompatibility between the donor and the patient cells as well as ethical issues related to the use of human embryos. Cells that reprogram by transferring somatic nuclear contents into oocytes or fusion with ES cells may overcome the tissue rejection problem following transplantation in patients, though not the ethical issues. As mentioned before, both these issues have recently been circumvented by the work of Takahashi and Yamanaka.[50] They have generated human pluripotent iPS cells directly from the patients' own cells, through retroviral transduction of a combination of specific transcription factors.[50,51] Moreover, a very recent study by Jaenisch's group strongly supports the therapeutic potential of this approach in hematological diseases.[158] Hematopoietic progenitors could be derived *in vitro* from iPS after infection with a viral vector encoding the HOXB4 product.[158] HOXB4 is a homeotic selector gene implicated in self-renewal of definitive HSCs, which also transforms primitive HSCs into definitive HSCs.[159] *In vitro* iPS-generated hematopoietic progenitor cells (HPCs) engraft adult recipient mice and convey multilineage reconstitution. Moreover, in a humanized mouse model of sickle cell anemia, transplanted iPS-derived HPC, corrected for the genetic defect by homologous recombination, functionally adjusted the sickle cell defect in donor mice.[158] These findings suggest that the therapeutic potential of iPS in several diseases is highly promising. However, as the authors themselves point out, difficulties related above all to the use of retroviral vectors for gene delivery and of oncogenes (that is Klf4 and c-Myc) for cell reprogramming will have to be overcome before this approach can be adopted as a patient-specific transplantation therapy.

HSC/HPC cell reprogramming

An interesting dual action is shared by a number of chromatin-remodeling agents, including RA, HDAC inhibitors (HDACi), valproic acid (VPA) and trichostatin A (TSA), and DNMT inhibitor (DNMTi) 5-azacytidine, as *in vitro* and *in vivo* studies performed in normal HSCs/HPCs have revealed: (1) expansion of a primitive HSC population, and (2) induction of myeloid

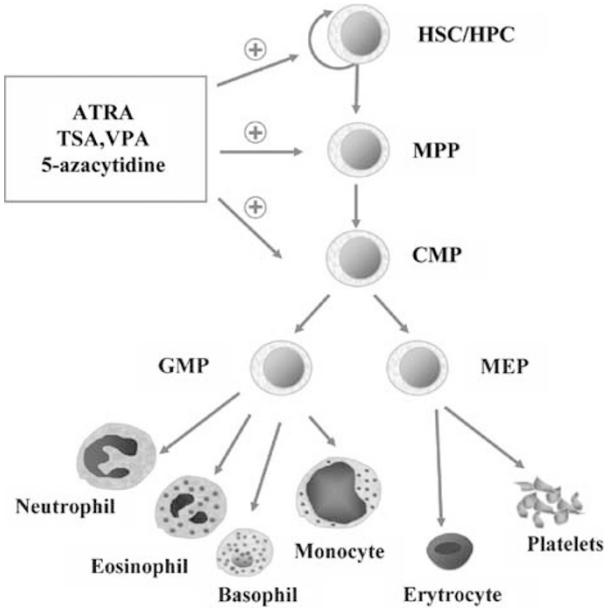

Figure 4 Dual action shared by different chromatin-remodeling agents in normal human hematopoietic cells. According to the model proposed in various *in vitro* and *in vivo* studies,[160–164] treatment with retinoic acid (RA), histone acetyl-deacetylase (HDAC) inhibitors (HDACi), valproic acid (VPA) and trichostatin A (TSA), and DNA methyltransferase (DNMT) inhibitor (DNMTi) 5-azacytidine all exert different effects on hematopoietic cells depending on their maturation state. They expand (+) primitive hematopoietic precursors (hematopoietic stem cell, HSC and multipotent progenitor, MPP) and enhance the terminal differentiation of committed myeloid progenitors (CMPs) into committed granulocyte/monocyte progenitors (GMPs) and megakaryocyte/erythroid progenitors (MEPs) and therefore into mature blood cells.

precursors committed to cell differentiation (schematically represented in Figure 4).[160–164] These findings support chromatin accessibility to specific DNA binding as a key event for the activity of cytokines and transcription factors involved either in the maintenance of early HSC population or in lineage commitment. Some of these factors might be present in the same cellular context and might dictate cell fate choice by targeting enzymes with chromatin-remodeling activity, such as HDACs, HATs or DNMTs, at specific gene loci. The marked effects of different chromatin-remodeling agents on human HSCs/HPCs also highlight their potential use as epigenetic agents for HSC *ex vivo* amplification aimed at transplantation, gene and stem cell therapies.

Leukemic cell reprogramming

The potential reversibility of epigenetic changes that contribute to the development of leukemia has suggested that it may be possible to reestablish normal patterns of gene expression and cell function by means of chromatin-remodeling agents. Proof of the effectiveness of this principle for epigenetic therapeutic purposes has also been provided by studies demonstrating the induction of terminal differentiation of PML/RARα-positive APL blasts[165,166] by all-*trans*-RA (ATRA) treatment *in vitro* and *in vivo*. By inhibiting HDAC and DNMTs activities, pharmacological doses of RA affect the chromatin and DNA methylation status at specific DNA binding sites on RA target gene promoters and induce terminal APL blast differentiation.[105,106,167–170] Indeed, the advent of retinoids combined with chemotherapy has led to a dramatic increase in the cure rate of this disease (up to 75% of cases).[165,171]

The antineoplastic efficacy of the considerably high number of molecules that specifically inhibit the function of different chromatin-modifying enzymes (listed in Tables 1–5) now available is currently being assessed in preclinical studies. However, some of these agents that possess DNMTi and/or HDACi activity have either been tested or are being tested in various clinical trials on myelodysplastic syndromes (MDSs) and AMLs, which we recently reviewed[172] and have summarized below.

DNMT inhibitors. Azanucleotides are cytidine analogs modified in position 5 of the pyrimidine ring in the presence of a nitrogen atom substituting a carbon. Azacytidine (5-azacytidine, Vidaza; Pharmion Corporation, Boulder, CO, USA) and decitabine (5-aza-2′-deoxycytydine, Dacogen; MGI Pharma Inc., Bloomington, MN, USA) are the drugs in this class that have been characterized the best. Both azacytidine and decitabine have now been approved by the United States Food and Drug Administration (FDA) for the treatment of MDS. Whereas, concentration of azacytidine and decitabine required for maximum inhibition of DNA methylation *in vitro* were not shown to suppress DNA synthesis, at high doses both azacytidine and decitabine are cytotoxic (like other cytidine analogs such as cytarabine).[173] At low doses, these demethylating agents maintain the ability to inhibit DNMTs and to cause DNA hypomethylation, thereby restoring the normal function of genes involved in the control of cellular proliferation and differentiation.[174] Indeed, large phase I, II and III trials have demonstrated that when used at a low-dose schedule (10–75 mg/m^2 per day), the clinical response is better in both AML and MDS.[172,175–178]

If compared with supportive care, both azacytidine and decitabine as single therapeutic agents display a high overall response rate, increased survival, improved quality of life and reduce the risk of leukemic transformation in MDS patients.[178–181] Decitabine, the more potent DNA-demethylating agent, resulted in 20–25% hematological complete response rate and a 31% of cytogenetic normalization rate when given intravenously for 72 h. Interestingly, cytogenetic normalization occurs more frequently in MDS with a 'poor risk' than 'intermediate risk' karyotype.[178,180,181]

However, the optimal azacytidine and decitabine schedules have yet to be defined, as do the molecular biomarkers of responsiveness to demethylating treatment, although no correlation has been observed between the response to azanucleotides and promoter demethylation of single genes, as *p15*.

Other compounds that exert demethylating activity, including 5-fluoro-2′-deoxycytidine, procaine, procainamide, hydralazine and (−)-epigallocatechin-3-gallate, have not been found to be nearly as effective as the first azanucleotides.[174,182,183]

HDAC inhibitors. Phenylbutyrate and the antiepileptic agent VPA are short-chain fatty acids that have been shown to possess, both *in vitro* and *in vivo*, the capacity to inhibit cell growth and to induce differentiation or apoptosis in solid and hematopoietic cancers.[184–187] When used as single agents in clinical trails for patients with MDS or AML, both these compounds displayed a low level of activity. However, when compared with phenylbutyrate, VPA was found to be an extremely safe, absorbable and well-tolerated drug.

The weak potency of short-chain fatty acids might be attributed to their inability to access the active catalytic pocket of HDACs. Therefore, to mediate enzyme inhibition, potent

HDAC inhibitors insert a long aliphatic chain into the tube-like active site, reaching the bottom of the pocket and allowing the chelating group to coordinate the Zn^{2+} ion. This strategy has led to a novel class of short-chain fatty acid derivatives that have markedly improved the inhibitory potency of HDAC; the best compounds were in the nanomolar range, the most potent in this novel class of HDACi being *N*-hydroxy-4-(4-phenyl-butyrylamino)-benzamide (HTBP).[188] The capability to bind Zn^{2+} might also be improved by using hydroxamic acid derivatives, which are significantly more effective than their carboxylic acid counterparts in providing active-site Zn chelation. The prototypes of this hydroxamic acid group are TSA and suberoylanilide hydroxamic acid (SAHA).[189–191] Dose-finding studies on treatments for hematopoietic tumors have been conducted on SAHA, which was found to possess good oral availability and favorable pharmacokinetics.[192] SAHA also displayed marked activity in patients with untreated, relapsed or refractory leukemias or MDS, Hodgkin's disease and certain subtypes of non-Hodgkin's lymphomas.[192,193] The FDA recently approved the clinical use of SAHA in the United States (Vorinostat) for cutaneous T-cell lymphomas on the basis of a phase II study with orally administered Vorinostat conducted on 33 previously treated patients with refractory cutaneous T-cell lymphoma. The results of that study showed a partial response in eight patients (24.2%) and the relief of pruritis in 14 out of 31 assessable patients (45.2%).[194]

Despite the high potency of hydroxamate analogs *in vitro*, such analogs might be metabolically unstable when administered *in vivo*. Many derivatives have, consequently, been designed and synthesized bearing alternative Zn^{2+} chelating groups.

Benzamides constitute another class of HDACi.[195] MS-275 is one of the compounds belonging to this class that has been selected for clinical trials.[196,197] Recent results from a phase I trial of orally administered MS-275 conducted on 38 adults with advanced acute leukemia have shown that MS-275 effectively inhibits HDAC *in vivo*. However, no clinical responses by classical criteria were observed.[196]

Lastly, HDACi based on a cyclopeptidic system include the most structurally complex molecules. With the exception of few examples, they are essentially constituted by a large cap group (typically a cyclic tetrapeptide containing hydrophobic amino acids) and a lateral chain ending with a chelating group. This class encompasses both irreversible and reversible HDACi, depending on whether or not they contain an epoxy end group. Though they exhibit low-nanomolar activity *in vitro*, their therapeutic potential has not yet been fully established. To date, only Apicidin (FK-228), which has documented proapoptotic, antiproliferative and antiangiogenic effects, is in phase II clinical trials.[198] Although *in vitro* evidence indicates that these compounds promote cell-growth arrest and differentiation of neoplastic cells at least as effectively as short-chain fatty acids, their therapeutic potential has yet to be fully determined. At least 14 different HDACi are currently being studied in clinical trials (see National Cancer Institute website for CTEP clinical trials (http://ctep.cancer.gov), the website of companies developing HDACi recently reported by Xu *et al*.).[199]

Combination drug regimens including HDACi and/or DNMTi

These reports taken as a whole indicate that although HDACi can markedly modify neoplastic cells in various ways, including growth arrest and cell differentiation, they do not exert a remarkable clinical activity when used as single agents. Thus, it is likely that HDACi will prove most useful as components of combination drug regimens. Several functional studies have recently focused on this issue, trying to identify the most effective drug combinations involving HDACi, and which cancer types might respond most to such combinations.

The clinical activity of VPA either alone or in combination with ATRA was first evaluated by Kuendgen *et al*.[200] on 23 MDS patients, in whom the overall response rate was 35%. A similar response rate was reported by the same group of researchers on an additional series of 58 patients with AML, including one patient, who developed an early relapse after an intensive chemotherapy course, in whom a durable CR lasting 16 months was achieved.[201]

We recently conducted a pilot study on eight refractory or high-risk AML patients not eligible for intensive therapy to assess the biological and therapeutic activities of the sequential combination of VPA, used to remodel chromatin, and ATRA, to activate gene transcription and differentiation in leukemic cells. The results of this study showed that global changes in the acetylation status of histones H3 and H4 in leukemic '*ex vivo*' cells from patients correlated both with VPA serum levels and the ability of such cells to undergo myelomonocytic differentiation. Differentiation of the leukemic clone was also proven by FISH analysis, which revealed the cytogenetic lesion +8 or 7q− in differentiating cells. Hematological improvement, according to the established criteria for MDS, was observed in two cases. Stable disease was observed in five cases and disease progression in one. VPA–ATRA was found to be a well-tolerated treatment that induced phenotypic maturation of AML blasts through chromatin remodeling.[202]

Moreover, VPA treatment was recently shown to maintain a significantly higher proportion of CD34+ leukemic progenitor cells (LPCs) and colony-forming units than control cultures in AML samples.[203] This raises the possibility that the effects of VPA exerts on the small population of AML progenitor cells may be different from those it exerts on the bulk of aberrantly differentiated AML blasts that represent the majority of the leukemia population. Treatment with VPA (and possibly with other chromatin-remodeling agents) might enhance the proliferation and self-renewal potential of LPCs, while generating a chromatin code reprogramming leukemic blasts harboring a block in their terminal differentiation. This hypothesis derives from the reported effect of chromatin-remodeling agents, including VPA in normal HSCs/HPCs, which was previously discussed in this review and schematically represented in Figure 3. Whether epigenetic changes in leukemic progenitors render AML blasts more sensitive to conventional chemotherapy or to novel therapeutic approaches (including other chromatin-remodeling agents) is a question that warrants further investigation.[160–164,203]

In view of the close functional correlation between chromatin histone changes and DNA methylation, clinical trials combining HDACi and DNMTi are being developed. The sequential administration of 5-azacytidine and sodium phenylbutyrate in patients with AML or MDS targeting different mechanisms has been found to be clinically feasible, with an acceptable level of toxicity and measurable biologic and clinical outcomes.[204] However, far more promising results have recently been reported by Garcia-Manero *et al*.[205] in a phase I–II study combining decitabine and VPA. In a restricted number (10 cases) of elderly patients with AML or MDS, they showed 50% of long-lasting CR associated with minimal toxicity. In a larger study performed in elderly and previously untreated

patients, the addition of ATRA to this regimen raised the response rate to 52%.[206]

Outlook

We believe that a point has been reached in which it may be possible to fully unravel the molecular mechanisms underlying the epigenetic regulation that directs embryonic and HSCs along the road of differentiation into several cell types, which would pave the way for the development of novel therapeutic approaches for hematological diseases. Moreover, the availability of several compounds that target different epigenetic-modifying activities offers the possibility to develop new treatment strategies in patients with hematopoietic malignancies that significantly raise the therapeutic effects, while reducing toxicity.

Acknowledgements

We thank all our past and current collaborators for expertize and discussions. We apologize that due to space limitations many crucial references were not directly cited but they can be found in cited reviews. This study was supported partially by grants from the Associazione Italiana per Ricerca sul Cancro (AIRC), Associazione Italiana contro le Leucemie Sezione di Roma (ROMAIL), 'La Sapienza' University of Rome and Italian Ministry for Universities and Research (MIUR).

References

1 Holliday R. The inheritance of epigenetic defects. *Science* 1987; **238**: 163–170.
2 Grewal SI, Moazed D. Heterochromatin and epigenetic control of gene expression. *Science* 2003; **301**: 798–802.
3 Lanzuolo C, Orlando V. The function of the epigenome in cell reprogramming. *Cell Mol Life Sci* 2007; **64**: 1043–1062.
4 Zaratiegui M, Irvine DV, Martienssen RA. Noncoding RNAs and gene silencing. *Cell* 2007; **128**: 763–776.
5 Turner BM. Defining an epigenetic code. *Nat Cell Biol* 2007; **9**: 2–6.
6 Downs JA, Nussenzweig MC, Nussenzweig A. Chromatin dynamics and the preservation of genetic information. *Nature* 2007; **447**: 951–958.
7 Fischle W, Wang Y, Allis CD. Histone and chromatin cross-talk. *Curr Opin Cell Biol* 2003; **15**: 172–183.
8 Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. *Annu Rev Biochem* 2005; **74**: 481–514.
9 Clark SJ. Action at a distance: epigenetic silencing of large chromosomal regions in carcinogenesis. *Hum Mol Genet* 2007; **16** (Spec No 1): R88–R95.
10 Miranda TB, Jones PA. DNA methylation: the nuts and bolts of repression. *J Cell Physiol* 2007; **213**: 384–390.
11 Okano M, Xie S, Li E. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. *Nat Genet* 1998; **19**: 219–220.
12 Aapola U, Lyle R, Krohn K, Antonarakis SE, Peterson P. Isolation and initial characterization of the mouse Dnmt3l gene. *Cytogenet Cell Genet* 2001; **92**: 122–126.
13 Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH. Dnmt3L and the establishment of maternal genomic imprints. *Science* 2001; **294**: 2536–2539.
14 Chedin F, Lieber MR, Hsieh CL. The DNA methyltransferase-like protein DNMT3L stimulates *de novo* methylation by Dnmt3a. *Proc Natl Acad Sci USA* 2002; **99**: 16916–16921.
15 Ooi SK, Qiu C, Bernstein E, Li K, Jia D, Yang Z *et al.* DNMT3L connects unmethylated lysine 4 of histone H3 to *de novo* methylation of DNA. *Nature* 2007; **448**: 714–717.
16 Yoo CB, Jones PA. Epigenetic therapy of cancer: past, present and future. *Nat Rev Drug Discov* 2006; **5**: 37–50.
17 Takai D, Jones PA. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. *Proc Natl Acad Sci USA* 2002; **99**: 3740–3745.
18 Smit AF, Riggs AD. Tiggers and DNA transposon fossils in the human genome. *Proc Natl Acad Sci USA* 1996; **93**: 1443–1448.
19 Kass SU, Landsberger N, Wolffe AP. DNA methylation directs a time-dependent repression of transcription initiation. *Curr Biol* 1997; **7**: 157–165.
20 Song F, Smith JF, Kimura MT, Morrow AD, Matsuyama T, Nagase H *et al.* Association of tissue-specific differentially methylated regions (TDMs) with differential gene expression. *Proc Natl Acad Sci USA* 2005; **102**: 3336–3341.
21 Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* 2006; **31**: 89–97.
22 Yang XJ. Lysine acetylation and the bromodomain: a new partnership for signaling. *Bioessays* 2004; **26**: 1076–1087.
23 Roth SY, Denu JM, Allis CD. Histone acetyltransferases. *Annu Rev Biochem* 2001; **70**: 81–120.
24 Gregoretti IV, Lee YM, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J Mol Biol* 2004; **338**: 17–31.
25 Berger SL. Histone modifications in transcriptional regulation. *Curr Opin Genet Dev* 2002; **12**: 142–148.
26 Kouzarides T. Histone methylation in transcriptional control. *Curr Opin Genet Dev* 2002; **12**: 198–209.
27 Bannister AJ, Kouzarides T. Histone methylation: recognizing the methyl mark. *Methods Enzymol* 2004; **376**: 269–288.
28 Kubicek S, O'Sullivan RJ, August EM, Hickey ER, Zhang Q, Teodoro ML *et al.* Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Mol Cell* 2007; **25**: 473–481.
29 Shi Y, Whetstine JR. Dynamic regulation of histone lysine methylation by demethylases. *Mol Cell* 2007; **25**: 1–14.
30 Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM *et al.* Control of developmental regulators by Polycomb in human embryonic stem cells. *Cell* 2006; **125**: 301–313.
31 Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP *et al.* Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* 2005; **122**: 947–956.
32 Schwartz YB, Pirrotta V. Polycomb silencing mechanisms and the management of genomic programmes. *Nat Rev Genet* 2007; **8**: 9–22.
33 Otte AP, Kwaks TH. Gene repression by Polycomb group protein complexes: a distinct complex for every occasion? *Curr Opin Genet Dev* 2003; **13**: 448–454.
34 Czermin B, Melfi R, McCabe D, Seitz V, Imhof A, Pirrotta V. Drosophila enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. *Cell* 2002; **111**: 185–196.
35 Fischle W, Wang Y, Jacobs SA, Kim Y, Allis CD, Khorasanizadeh S. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. *Genes Dev* 2003; **17**: 1870–1881.
36 Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS *et al.* Role of histone H2A ubiquitination in Polycomb silencing. *Nature* 2004; **431**: 873–878.
37 Dellino GI, Schwartz YB, Farkas G, McCabe D, Elgin SC, Pirrotta V. Polycomb silencing blocks transcription initiation. *Mol Cell* 2004; **13**: 887–893.
38 Francis NJ, Kingston RE. Mechanisms of transcriptional memory. *Nat Rev Mol Cell Biol* 2001; **2**: 409–421.
39 Daujat S, Zeissler U, Waldmann T, Happel N, Schneider R. HP1 binds specifically to Lys26-methylated histone H1.4, whereas simultaneous Ser27 phosphorylation blocks HP1 binding. *J Biol Chem* 2005; **280**: 38090–38095.
40 Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C *et al.* The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* 2006; **439**: 871–874.
41 Mohd-Sarip A, van der Knaap JA, Wyman C, Kanaar R, Schedl P, Verrijzer CP. Architecture of a polycomb nucleoprotein complex. *Mol Cell* 2006; **24**: 91–100.

42 Beisel C, Imhof A, Greene J, Kremmer E, Sauer F. Histone methylation by the *Drosophila* epigenetic transcriptional regulator Ash1. *Nature* 2002; **419**: 857–862.
43 Xia ZB, Anderson M, Diaz MO, Zeleznik L. MLL repression domain interacts with histone deacetylases, the polycomb group proteins HPC2 and BMI-1, and the corepressor C-terminal-binding protein. *Proc Natl Acad Sci USA* 2003; **100**: 8342–8347.
44 Ernst P, Mabon M, Davidson AJ, Zon LI, Korsmeyer SJ. An Mll-dependent Hox program drives hematopoietic progenitor expansion. *Curr Biol* 2004; **14**: 2063–2069.
45 Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, Allis CD *et al.* MLL targets SET domain methyltransferase activity to Hox gene promoters. *Mol Cell* 2002; **10**: 1107–1117.
46 Dou Y, Milne TA, Tackett AJ, Smith ER, Fukuda A, Wysocka J *et al.* Physical association and coordinate function of the H3 K4 methyltransferase MLL1 and the H4 K16 acetyltransferase MOF. *Cell* 2005; **121**: 873–885.
47 Milne TA, Martin ME, Brock HW, Slany RK, Hess JL. Leukemogenic MLL fusion proteins bind across a broad region of the Hox a9 locus, promoting transcription and multiple histone modifications. *Cancer Res* 2005; **65**: 11367–11374.
48 Guenther MG, Jenner RG, Chevalier B, Nakamura T, Croce CM, Canaani E *et al.* Global and Hox-specific roles for the MLL1 methyltransferase. *Proc Natl Acad Sci USA* 2005; **102**: 8603–8608.
49 Niwa H. How is pluripotency determined and maintained? *Development* 2007; **134**: 635–646.
50 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663–676.
51 Takahashi K, Okita K, Nakagawa M, Yamanaka S. Induction of pluripotent stem cells from fibroblast cultures. *Nat Protoc* 2007; **2**: 3081–3089.
52 Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell* 2008; **132**: 567–582.
53 Meshorer E, Misteli T. Chromatin in pluripotent embryonic stem cells and differentiation. *Nat Rev Mol Cell Biol* 2006; **7**: 540–546.
54 Brown DT. Histone H1 and the dynamic regulation of chromatin function. *Biochem Cell Biol* 2003; **81**: 221–227.
55 Phair RD, Scaffidi P, Elbi C, Vecerova J, Dey A, Ozato K *et al.* Global nature of dynamic protein-chromatin interactions *in vivo*: three-dimensional genome scanning and dynamic interaction networks of chromatin proteins. *Mol Cell Biol* 2004; **24**: 6393–6402.
56 Wiblin AE, Cui W, Clark AJ, Bickmore WA. Distinctive nuclear organisation of centromeres and regions involved in pluripotency in human embryonic stem cells. *J Cell Sci* 2005; **118**: 3861–3868.
57 Lee JH, Hart SR, Skalnik DG. Histone deacetylase activity is required for embryonic stem cell differentiation. *Genesis* 2004; **38**: 32–38.
58 Azuara V, Perry P, Sauer S, Spivakov M, Jorgensen HF, John RM *et al.* Chromatin signatures of pluripotent cell lines. *Nat Cell Biol* 2006; **8**: 532–538.
59 Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J *et al.* A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 2006; **125**: 315–326.
60 Guccione E, Martinato F, Finocchiaro G, Luzi L, Tizzoni L, Dall O *et al.* Myc-binding-site recognition in the human genome is determined by chromatin context. *Nat Cell Biol* 2006; **8**: 764–770.
61 Jackson-Grusby L, Beard C, Possemato R, Tudor M, Fambrough D, Csankovszki G *et al.* Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. *Nat Genet* 2001; **27**: 31–39.
62 Jackson M, Krassowska A, Gilbert N, Chevassut T, Forrester L, Ansell J *et al.* Severe global DNA hypomethylation blocks differentiation and induces histone hyperacetylation in embryonic stem cells. *Mol Cell Biol* 2004; **24**: 8862–8871.
63 Blelloch R, Wang Z, Meissner A, Pollard S, Smith A, Jaenisch R. Reprogramming efficiency following somatic cell nuclear transfer is influenced by the differentiation and methylation state of the donor nucleus. *Stem Cells* 2006; **24**: 2007–2013.
64 Goren A, Cedar H. Replicating by the clock. *Nat Rev Mol Cell Biol* 2003; **4**: 25–32.
65 Perry P, Sauer S, Billon N, Richardson WD, Spivakov M, Warnes G *et al.* A dynamic switch in the replication timing of key regulator genes in embryonic stem cells upon neural induction. *Cell Cycle* 2004; **3**: 1645–1650.
66 Williams RR, Azuara V, Perry P, Sauer S, Dvorkina M, Jorgensen H *et al.* Neural induction promotes large-scale chromatin reorganisation of the Mash1 locus. *J Cell Sci* 2006; **119**: 132–140.
67 Ohm JE, McGarvey KM, Yu X, Cheng L, Schuebel KE, Cope L *et al.* A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. *Nat Genet* 2007; **39**: 237–242.
68 Plath K, Fang J, Mlynarczyk-Evans SK, Cao R, Worringer KA, Wang H *et al.* Role of histone H3 lysine 27 methylation in X inactivation. *Science* 2003; **300**: 131–135.
69 Koyanagi M, Baguet A, Martens J, Margueron R, Jenuwein T, Bix M. EZH2 and histone 3 trimethyl lysine 27 associated with Il4 and Il13 gene silencing in Th1 cells. *J Biol Chem* 2005; **280**: 31470–31477.
70 Nakayama J, Rice JC, Strahl BD, Allis CD, Grewal SI. Role of histone H3 lysine 9 methylation in epigenetic control of heterochromatin assembly. *Science* 2001; **292**: 110–113.
71 Jiang G, Yang F, Sanchez C, Ehrlich M. Histone modification in constitutive heterochromatin versus unexpressed euchromatin in human cells. *J Cell Biochem* 2004; **93**: 286–300.
72 Boyer LA, Plath K, Zeitlinger J, Brambrink T, Medeiros LA, Lee TI *et al.* Polycomb complexes repress developmental regulators in murine embryonic stem cells. *Nature* 2006; **441**: 349–353.
73 Bracken AP, Dietrich N, Pasini D, Hansen KH, Helin K. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. *Genes Dev* 2006; **20**: 1123–1136.
74 Sparmann A, van Lohuizen M. Polycomb silencers control cell fate, development and cancer. *Nat Rev Cancer* 2006; **6**: 846–856.
75 Akashi K. Lineage promiscuity and plasticity in hematopoietic development. *Ann N Y Acad Sci* 2005; **1044**: 125–131.
76 Zhu J, Emerson SP. Hematopoietic cytokines, transcription factors and lineage commitment. *Oncogene* 2002; **21**: 3295–3313.
77 Mikkola HK, Orkin SH. The journey of developing hematopoietic stem cells. *Development* 2006; **133**: 3733–3744.
78 Tenen DG. Disruption of differentiation in human cancer: AML shows the way. *Nat Rev Cancer* 2003; **3**: 89–101.
79 Bonifer C. Epigenetic plasticity of hematopoietic cells. *Cell Cycle* 2005; **4**: 211–214.
80 Escamilla-Del-Arenal M, Recillas-Targa F. GATA-1 modulates the chromatin structure and activity of the chicken alpha-globin 3′ enhancer. *Mol Cell Biol* 2008; **28**: 575–586.
81 Layon ME, Ackley CJ, West RJ, Lowrey CH. Expression of GATA-1 in a non-hematopoietic cell line induces beta-globin locus control region chromatin structure remodeling and an erythroid pattern of gene expression. *J Mol Biol* 2007; **366**: 737–744.
82 Levings PP, Zhou Z, Vieira KF, Crusselle-Davis VJ, Bungert J. Recruitment of transcription complexes to the beta-globin locus control region and transcription of hypersensitive site 3 prior to erythroid differentiation of murine embryonic stem cells. *FEBS J* 2006; **273**: 746–755.
83 Attema JL, Papathanasiou P, Forsberg EC, Xu J, Smale ST, Weissman IL. Epigenetic characterization of hematopoietic stem cell differentiation using miniChIP and bisulfite sequencing analysis. *Proc Natl Acad Sci USA* 2007; **104**: 12371–12376.
84 Stopka T, Amanatullah DF, Papetti M, Skoultchi AI. PU.1 inhibits the erythroid program by binding to GATA-1 on DNA and creating a repressive chromatin structure. *EMBO J* 2005; **24**: 3712–3723.
85 Huang S, Brandt SJ. mSin3A regulates murine erythroleukemia cell differentiation through association with the TAL1 (or SCL) transcription factor. *Mol Cell Biol* 2000; **20**: 2248–2259.
86 Friedman AD. Transcriptional control of granulocyte and monocyte development. *Oncogene* 2007; **26**: 6816–6828.
87 Ivascu C, Wasserkort R, Lesche R, Dong J, Stein H, Thiel A *et al.* DNA methylation profiling of transcription factor genes in normal lymphocyte development and lymphomas. *Int J Biochem Cell Biol* 2007; **39**: 1523–1538.
88 Tatetsu H, Ueno S, Hata H, Yamada Y, Takeya M, Mitsuya H *et al.* Down-regulation of PU.1 by methylation of distal regulatory elements and the promoter is required for myeloma cell growth. *Cancer Res* 2007; **67**: 5328–5336.

89 Sherr CJ. Colony-stimulating factor-1 receptor. *Blood* 1990; **75**: 1–12.
90 Bonifer C, Hume DA. The transcriptional regulation of the colony-stimulating factor 1 receptor (csf1r) gene during hematopoiesis. *Front Biosci* 2008; **13**: 549–560.
91 Krysinska H, Hoogenkamp M, Ingram R, Wilson N, Tagoh H, Laslo P *et al.* A two-step, PU.1-dependent mechanism for developmentally regulated chromatin remodeling and transcription of the c-fms gene. *Mol Cell Biol* 2007; **27**: 878–887.
92 Plachetka A, Chayka O, Wilczek C, Melnik S, Bonifer C, Klempnauer KH. C/EBPbeta induces chromatin opening at a cell-type-specific enhancer. *Mol Cell Biol* 2008; **28**: 2102–2112.
93 Rosenbauer F, Tenen DG. Transcription factors in myeloid development: balancing differentiation with transformation. *Nat Rev Immunol* 2007; **7**: 105–117.
94 Jones PA, Baylin SB. The epigenomics of cancer. *Cell* 2007; **128**: 683–692.
95 Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007; **8**: 286–298.
96 Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR *et al.* Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French–American–British Cooperative Group. *Ann Intern Med* 1985; **103**: 620–625.
97 Moe-Behrens GH, Pandolfi PP. Targeting aberrant transcriptional repression in acute myeloid leukemia. *Rev Clin Exp Hematol* 2003; **7**: 139–159.
98 Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer* 2006; **6**: 38–51.
99 Di Croce L. Chromatin modifying activity of leukaemia associated fusion proteins. *Hum Mol Genet* 2005; **14** (Spec No 1): R77–R84.
100 Melnick A. Predicting the effect of transcription therapy in hematologic malignancies. *Leukemia* 2005; **19**: 1109–1117.
101 Glass CK, Rosenfeld MG. The coregulator exchange in transcriptional functions of nuclear receptors. *Genes Dev* 2000; **14**: 121–141.
102 Chambon P. A decade of molecular biology of retinoic acid receptors. *FASEB J* 1996; **10**: 940–954.
103 Villa R, Morey L, Raker VA, Buschbeck M, Gutierrez A, De Santis F *et al.* The methyl-CpG binding protein MBD1 is required for PML-RARalpha function. *Proc Natl Acad Sci USA* 2006; **103**: 1400–1405.
104 Carbone R, Botrugno OA, Ronzoni S, Insinga A, Di Croce L, Pelicci PG *et al.* Recruitment of the histone methyltransferase SUV39H1 and its role in the oncogenic properties of the leukemia-associated PML-retinoic acid receptor fusion protein. *Mol Cell Biol* 2006; **26**: 1288–1296.
105 Fazi F, Zardo G, Gelmetti V, Travaglini L, Ciolfi A, Di Croce L *et al.* Heterochromatic gene repression of the retinoic acid pathway in acute myeloid leukemia. *Blood* 2007; **109**: 4432–4440.
106 Di Croce L, Raker VA, Corsaro M, Fazi F, Fanelli M, Faretta M *et al.* Methyltransferase recruitment and DNA hypermethylation of target promoters by an oncogenic transcription factor. *Science* 2002; **295**: 1079–1082.
107 Villa R, Pasini D, Gutierrez A, Morey L, Occhionorelli M, Vire E *et al.* Role of the polycomb repressive complex 2 in acute promyelocytic leukemia. *Cancer Cell* 2007; **11**: 513–525.
108 Zeisig BB, Kwok C, Zelent A, Shankaranarayanan P, Gronemeyer H, Dong S *et al.* Recruitment of RXR by homotetrameric RARalpha fusion proteins is essential for transformation. *Cancer Cell* 2007; **12**: 36–51.
109 Zhu J, Nasr R, Peres L, Riaucoux-Lormiere F, Honore N, Berthier C *et al.* RXR is an essential component of the oncogenic PML/RARA complex *in vivo*. *Cancer Cell* 2007; **12**: 23–35.
110 Hiebert SW, Downing JR, Lenny N, Meyers S. Transcriptional regulation by the *t*(8;21) fusion protein, AML-1/ETO. *Curr Top Microbiol Immunol* 1996; **211**: 253–258.
111 Look AT. Oncogenic transcription factors in the human acute leukemias. *Science* 1997; **278**: 1059–1064.
112 Nucifora G, Birn DJ, Erickson P, Gao J, LeBeau MM, Drabkin HA *et al.* Detection of DNA rearrangements in the AML1 and ETO loci and of an AML1/ETO fusion mRNA in patients with *t*(8;21) acute myeloid leukemia. *Blood* 1993; **81**: 883–888.
113 Peterson LF, Zhang DE. The 8;21 translocation in leukemogenesis. *Oncogene* 2004; **23**: 4255–4262.
114 Pabst T, Mueller BU, Harakawa N, Schoch C, Haferlach T, Behre G *et al.* AML1-ETO downregulates the granulocytic differentiation factor C/EBPalpha in *t*(8;21) myeloid leukemia. *Nat Med* 2001; **7**: 444–451.
115 Fazi F, Zardo G, Gelmetti V, Travaglini L, Ciolfi A, Di Croce L *et al.* Heterochromatic gene repression of the retinoic acid pathway in acute myeloid leukemia. *Blood* 2007; **109**: 4432–4440.
116 Choi Y, Elagib KE, Delehanty LL, Goldfarb AN. Erythroid inhibition by the leukemic fusion AML1-ETO is associated with impaired acetylation of the major erythroid transcription factor GATA-1. *Cancer Res* 2006; **66**: 2990–2996.
117 Zhang J, Kalkum M, Yamamura S, Chait BT, Roeder RG. E protein silencing by the leukemogenic AML1-ETO fusion protein. *Science* 2004; **305**: 1286–1289.
118 Fazi F, Racanicchi S, Zardo G, Starnes LM, Mancini M, Travaglini L *et al.* Epigenetic silencing of the myelopoiesis regulator microRNA-223 by the AML1/ETO oncoprotein. *Cancer Cell* 2007; **12**: 457–466.
119 Nervi C, Fazi F, Grignani F. Oncoproteins, heterochromatin silencing and microRNAs: a new link for leukemogenesis. *Epigenetics* 2008; **3**: 1–4.
120 Alcalay M, Meani N, Gelmetti V, Fantozzi A, Fagioli M, Orleth A *et al.* Acute myeloid leukemia fusion proteins deregulate genes involved in stem cell maintenance and DNA repair. *J Clin Invest* 2003; **112**: 1751–1761.
121 Hess JL, Hug BA. Fusion-protein truncation provides new insights into leukemogenesis. *Proc Natl Acad Sci USA* 2004; **101**: 16985–16986.
122 Rozman M, Camos M, Colomer D, Villamor N, Esteve J, Costa D *et al.* Type I MOZ/CBP (MYST3/CREBBP) is the most common chimeric transcript in acute myeloid leukemia with *t*(8;16)(p11;p13) translocation. *Genes Chromosomes Cancer* 2004; **40**: 140–145.
123 Panagopoulos I, Fioretos T, Isaksson M, Samuelsson U, Billstrom R, Strombeck B *et al.* Fusion of the MORF and CBP genes in acute myeloid leukemia with the *t*(10;16)(q22;p13). *Hum Mol Genet* 2001; **10**: 395–404.
124 Deguchi K, Ayton PM, Carapeti M, Kutok JL, Snyder CS, Williams IR *et al.* MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of CBP. *Cancer Cell* 2003; **3**: 259–271.
125 Troke PJ, Kindle KB, Collins HM, Heery DM. MOZ fusion proteins in acute myeloid leukaemia. *Biochem Soc Symp* 2006; **73**: 23–39.
126 Peters AH, O'Carroll D, Scherthan H, Mechtler K, Sauer S, Schofer C *et al.* Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell* 2001; **107**: 323–337.
127 Braig M, Lee S, Loddenkemper C, Rudolph C, Peters AH, Schlegelberger B *et al.* Oncogene-induced senescence as an initial barrier in lymphoma development. *Nature* 2005; **436**: 660–665.
128 Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM *et al.* hDOT1L links histone methylation to leukemogenesis. *Cell* 2005; **121**: 167–178.
129 Widschwendter M, Fiegl H, Egle D, Mueller-Holzner E, Spizzo G, Marth C *et al.* Epigenetic stem cell signature in cancer. *Nat Genet* 2007; **39**: 157–158.
130 Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, Zimmerman J *et al.* Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for *de novo* methylation in cancer. *Nat Genet* 2007; **39**: 232–236.
131 Kirmizis A, Bartley SM, Farnham PJ. Identification of the polycomb group protein SU(Z)12 as a potential molecular target for human cancer therapy. *Mol Cancer Ther* 2003; **2**: 113–121.
132 Van Kemenade FJ, Raaphorst FM, Blokzijl T, Fieret E, Hamer KM, Satijn DP *et al.* Coexpression of BMI-1 and EZH2 polycomb-group proteins is associated with cycling cells and degree of malignancy in B-cell non-Hodgkin lymphoma. *Blood* 2001; **97**: 3896–3901.
133 Richie ER, Schumacher A, Angel JM, Holloway M, Rinchik EM, Magnuson T. The Polycomb-group gene eed regulates thymocyte

differentiation and suppresses the development of carcinogen-induced T-cell lymphomas. *Oncogene* 2002; **21**: 299–306.
134 Chowdhury M, Mihara K, Yasunaga S, Ohtaki M, Takihara Y, Kimura A. Expression of Polycomb-group (PcG) protein BMI-1 predicts prognosis in patients with acute myeloid leukemia. *Leukemia* 2007; **21**: 1116–1122.
135 Mohty M, Yong AS, Szydlo RM, Apperley JF, Melo JV. The polycomb group BMI1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia. *Blood* 2007; **110**: 380–383.
136 Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* 2003; **423**: 255–260.
137 Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL *et al.* Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* 2003; **423**: 302–305.
138 Jacobs JJ, Kieboom K, Marino S, dePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature* 1999; **397**: 164–168.
139 Jacobs JJ, Scheijen B, Voncken JW, Kieboom K, Berns A, van Lohuizen M. Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. *Genes Dev* 1999; **13**: 2678–2690.
140 Biondi A, Cimino G, Pieters R, Pui CH. Biological and therapeutic aspects of infant leukemia. *Blood* 2000; **96**: 24–33.
141 Cimino G, Moir DT, Canaani O, Williams K, Crist WM, Katzav S *et al.* Cloning of ALL-1, the locus involved in leukemias with the *t*(4;11)(q21;q23), *t*(9;11)(p22;q23), and *t*(11;19)(q23;p13) chromosome translocations. *Cancer Res* 1991; **51**: 6712–6714.
142 Hess JL. MLL: a histone methyltransferase disrupted in leukemia. *Trends Mol Med* 2004; **10**: 500–507.
143 Ida K, Kitabayashi I, Taky T, Taniwaki M, Novo K, Yamamoto M *et al.* Adenovirus E1A-associated protein p300 is involved in acute myeloid leukaemia with *t*(11;22). *Blood* 1997; **90**: 4699–4704.
144 Linggi B, Muller-Tidow C, van de Locht L, Hu M, Nip J, Serve H *et al.* The *t*(8;21) fusion protein, AML1 ETO, specifically represses the transcription of the p14(ARF) tumor suppressor in acute myeloid leukemia. *Nat Med* 2002; **8**: 743–750.
145 Rowley JD, Reshmi S, Sobulo O, Musvee T, Anastasi J, Raimondi S *et al.* All patients with the T(11;16)(q23;p13.3) that involves MLL and CBP have treatment-related hematologic disorders. *Blood* 1997; **90**: 535–541.
146 Dorrance AM, Liu S, Yuan W, Becknell B, Arnoczky KJ, Guimond M *et al.* Mll partial tandem duplication induces aberrant Hox expression *in vivo* via specific epigenetic alterations. *J Clin Invest* 2006; **116**: 2707–2716.
147 Megonigal MD, Cheung NK, Rappaport EF, Nowell PC, Wilson RB, Jones DH *et al.* Detection of leukemia-associated MLL-GAS7 translocation early during chemotherapy with DNA topoisomerase II inhibitors. *Proc Natl Acad Sci USA* 2000; **97**: 2814–2819.
148 Bernard OA, Mauchauffe M, Mecucci C, Van den BH, Berger R. A novel gene, AF-1p, fused to HRX in *t*(1;11)(p32;q23), is not related to AF-4, AF-9 nor ENL. *Oncogene* 1994; **9**: 1039–1045.
149 So CW, Lin M, Ayton PM, Chen EH, Cleary ML. Dimerization contributes to oncogenic activation of MLL chimeras in acute leukemias. *Cancer Cell* 2003; **4**: 99–110.
150 So CW, Karsunky H, Passegue E, Cozzio A, Weissman IL, Cleary ML. MLL-GAS7 transforms multipotent hematopoietic progenitors and induces mixed lineage leukemias in mice. *Cancer Cell* 2003; **3**: 161–171.
151 So CW, Cleary ML. Common mechanism for oncogenic activation of MLL by forkhead family proteins. *Blood* 2003; **101**: 633–639.
152 Erfurth F, Hemenway CS, de Erkenez AC, Domer PH. MLL fusion partners AF4 and AF9 interact at subnuclear foci. *Leukemia* 2004; **18**: 92–102.
153 Zeisig DT, Bittner CB, Zeisig BB, Garcia-Cuellar MP, Hess JL, Slany RK. The eleven-nineteen-leukemia protein ENL connects nuclear MLL fusion partners with chromatin. *Oncogene* 2005; **24**: 5525–5532.
154 Bitoun E, Oliver PL, Davies KE. The mixed-lineage leukemia fusion partner AF4 stimulates RNA polymerase II transcriptional elongation and mediates coordinated chromatin remodeling. *Hum Mol Genet* 2007; **16**: 92–106.
155 Zeisig BB, Milne T, Garcia-Cuellar MP, Schreiner S, Martin ME, Fuchs U *et al.* Hoxa9 and Meis1 are key targets for MLL-ENL-mediated cellular immortalization. *Mol Cell Biol* 2004; **24**: 617–628.
156 Kroon E, Krosl J, Thorsteinsdottir U, Baban S, Buchberg AM, Sauvageau G. Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. *EMBO J* 1998; **17**: 3714–3725.
157 Moskow JJ, Bullrich F, Huebner K, Daar IO, Buchberg AM. Meis1, a PBX1-related homeobox gene involved in myeloid leukemia in BXH-2 mice. *Mol Cell Biol* 1995; **15**: 5434–5443.
158 Hanna J, Wernig M, Markoulaki S, Sun CW, Meissner A, Cassady JP *et al.* Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 2007; **318**: 1920–1923.
159 Kyba M, Perlingeiro RC, Daley GQ. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. *Cell* 2002; **109**: 29–37.
160 Purton LE, Bernstein ID, Collins SJ. All-*trans* retinoic acid delays the differentiation of primitive hematopoietic precursors (lin-c-kit+Sca-1(+)) while enhancing the terminal maturation of committed granulocyte/monocyte progenitors. *Blood* 1999; **94**: 483–495.
161 De Felice L, Tatarelli C, Mascolo MG, Gregorj C, Agostini F, Fiorini R *et al.* Histone deacetylase inhibitor valproic acid enhances the cytokine-induced expansion of human hematopoietic stem cells. *Cancer Res* 2005; **65**: 1505–1513.
162 Milhem M, Mahmud N, Lavelle D, Araki H, DeSimone J, Saunthararajah Y *et al.* Modification of hematopoietic stem cell fate by 5aza 2′deoxycytidine and trichostatin A. *Blood* 2004; **103**: 4102–4110.
163 Bug G, Gul H, Schwarz K, Pfeifer H, Kampfmann M, Zheng X *et al.* Valproic acid stimulates proliferation and self-renewal of hematopoietic stem cells. *Cancer Res* 2005; **65**: 2537–2541.
164 Purton LE, Bernstein ID, Collins SJ. All-*trans* retinoic acid enhances the long-term repopulating activity of cultured hematopoietic stem cells. *Blood* 2000; **95**: 470–477.
165 Melnick A, Licht JD. Deconstructing a disease: RARα, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood* 1999; **93**: 3167–3215.
166 Warrell Jr RP, Frankel SR, Miller WHJ, Scheinberg DA, Itri LM, Hittelman WN *et al.* Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans*-retinoic acid). *N Engl J Med* 1991; **324**: 1385–1393.
167 Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M *et al.* Fusion proteins of the retinoic acid receptor-α recruit histone deacetylase in promyelocytic leukaemia. *Nature* 1998; **391**: 815–818.
168 Lin RJ, Nagy L, Inoue S, Shao W, Miller WHJ, Evans RM. Role of the histone deacetylase complex in acute promyelocytic leukaemia. *Nature* 1998; **391**: 811–814.
169 He LZ, Guidez F, Tribioli C, Peruzzi D, Ruthardt M, Zelent A *et al.* Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. *Nat Genet* 1998; **18**: 126–135.
170 He LI, Tribioli C, Rivi R, Peruzzi D, Pelicci PG, Soares V *et al.* Acute leukemia with promyelocytic features in PML/RARα transgenic mice. *Proc Natl Acad Sci USA* 1997; **94**: 5302–5307.
171 Sanz MA, Tallman MS, Lo-Coco F. Tricks of the trade for the appropriate management of newly diagnosed acute promyelocytic leukemia. *Blood* 2005; **105**: 3019–3025.
172 Leone G, D'alò F, Zardo G, Voso MT, Nervi C. Epigenetic treatment of myelodysplastic syndromes and acute myeloid leukemias. *Curr Med Chem* 2008; **15**: 1274–1287.
173 Saiki JH, Bodey GP, Hewlett JS, Amare M, Morrison FS, Wilson HE *et al.* Effect of schedule on activity and toxicity of 5-azacytidine in acute leukemia: a Southwest Oncology Group Study. *Cancer* 1981; **47**: 1739–1742.
174 Muller CI, Ruter B, Koeffler HP, Lubbert M. DNA hypermethylation of myeloid cells, a novel therapeutic target in MDS and AML. *Curr Pharm Biotechnol* 2006; **7**: 315–321.
175 Silverman LR, McKenzie DR, Peterson BL, Holland JF, Backstrom JT, Beach CL *et al.* Further analysis of trials with azacitidine in

patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. *J Clin Oncol* 2006; **24**: 3895–3903.
176 Sudan N, Rossetti JM, Shadduck RK, Latsko J, Lech JA, Kaplan RB *et al.* Treatment of acute myelogenous leukemia with outpatient azacitidine. *Cancer* 2006; **107**: 1839–1843.
177 Issa JP, Garcia-Manero G, Giles FJ, Mannari R, Thomas D, Faderl S *et al.* Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2′-deoxycytidine (decitabine) in hematopoietic malignancies. *Blood* 2004; **103**: 1635–1640.
178 Wijermans PW, Lubbert M, Verhoef G, Klimek V, Bosly A. An epigenetic approach to the treatment of advanced MDS; the experience with the DNA demethylating agent 5-aza-2′-deoxycytidine (decitabine) in 177 patients. *Ann Hematol* 2005; **84** (Suppl 1): 9–17.
179 Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R *et al.* Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. *J Clin Oncol* 2002; **20**: 2429–2440.
180 Wijermans P, Lubbert M, Verhoef G, Bosly A, Ravoet C, Andre M *et al.* Low-dose 5-aza-2′-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: a multicenter phase II study in elderly patients. *J Clin Oncol* 2000; **18**: 956–962.
181 Lubbert M, Wijermans P, Kunzmann R, Verhoef G, Bosly A, Ravoet C *et al.* Cytogenetic responses in high-risk myelodysplastic syndrome following low-dose treatment with the DNA methylation inhibitor 5-aza-2′-deoxycytidine. *Br J Haematol* 2001; **114**: 349–357.
182 Esteller M. DNA methylation and cancer therapy: new developments and expectations. *Curr Opin Oncol* 2005; **17**: 55–60.
183 Lubbert M. DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action. *Curr Top Microbiol Immunol* 2000; **249**: 135–164.
184 Johnstone RW. Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. *Nat Rev Drug Discov* 2002; **1**: 287–299.
185 Gottlicher M, Minucci S, Zhu P, Kramer OH, Schimpf A, Giavara S *et al.* Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J* 2001; **20**: 6969–6978.
186 Nebbioso A, Clarke N, Voltz E, Germain E, Ambrosino C, Bontempo P *et al.* Tumor-selective action of HDAC inhibitors involves TRAIL induction in acute myeloid leukemia cells. *Nat Med* 2005; **11**: 77–84.
187 Insinga A, Monestiroli S, Ronzoni S, Gelmetti V, Marchesi F, Viale A *et al.* Inhibitors of histone deacetylases induce tumor-selective apoptosis through activation of the death receptor pathway. *Nat Med* 2005; **11**: 71–76.
188 Lu Q, Yang YT, Chen CS, Davis M, Byrd JC, Etherton MR *et al.* Zn2+-chelating motif-tethered short-chain fatty acids as a novel class of histone deacetylase inhibitors. *J Med Chem* 2004; **47**: 467–474.
189 Yoshida M, Horinouchi S, Beppu T. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *Bioessays* 1995; **17**: 423–430.
190 Richon VM, Emiliani S, Verdin E, Webb Y, Breslow R, Rifkind RA *et al.* A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. *Proc Natl Acad Sci USA* 1998; **95**: 3003–3007.
191 Yoshida M, Kijima M, Akita M, Beppu T. Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. *J Biol Chem* 1990; **265**: 17174–17179.
192 O'Connor OA, Heaney ML, Schwartz L, Richardson S, Willim R, MacGregor-Cortelli B *et al.* Clinical experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. *J Clin Oncol* 2006; **24**: 166–173.
193 Garcia-Manero G, Yang H, Bueso-Ramos C, Ferrajoli A, Cortes J, Wierda WG *et al.* Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes. *Blood* 2008; **111**: 1060–1066.
194 Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C *et al.* Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). *Blood* 2007; **109**: 31–39.
195 Suzuki T, Ando T, Tsuchiya K, Fukazawa N, Saito A, Mariko Y *et al.* Synthesis and histone deacetylase inhibitory activity of new benzamide derivatives. *J Med Chem* 1999; **42**: 3001–3003.
196 Gojo I, Jiemjit A, Trepel JB, Sparreboom A, Figg WD, Rollins S *et al.* Phase 1 and pharmacologic study of MS-275, a histone deacetylase inhibitor, in adults with refractory and relapsed acute leukemias. *Blood* 2007; **109**: 2781–2790.
197 Ryan QC, Headlee D, Acharya M, Sparreboom A, Trepel JB, Ye J *et al.* Phase I and pharmacokinetic study of MS-275, a histone deacetylase inhibitor, in patients with advanced and refractory solid tumors or lymphoma. *J Clin Oncol* 2005; **23**: 3912–3922.
198 Singh SB, Zink DL, Liesch JM, Mosley RT, Dombrowski AW, Bills GF *et al.* Structure and chemistry of apicidins, a class of novel cyclic tetrapeptides without a terminal alpha-keto epoxide as inhibitors of histone deacetylase with potent antiprotozoal activities. *J Org Chem* 2002; **67**: 815–825.
199 Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* 2007; **26**: 5541–5552.
200 Kuendgen A, Strupp C, Aivado M, Bernhardt A, Hildebrandt B, Haas R *et al.* Treatment of myelodysplastic syndromes with valproic acid alone or in combination with all-trans retinoic acid. *Blood* 2004; **104**: 1266–1269.
201 Kuendgen A, Knipp S, Fox F, Strupp C, Hildebrandt B, Steidl C *et al.* Results of a phase 2 study of valproic acid alone or in combination with all-trans retinoic acid in 75 patients with myelodysplastic syndrome and relapsed or refractory acute myeloid leukemia. *Ann Hematol* 2005; **84** (Suppl 13): 61–66.
202 Cimino G, Lo-Coco F, Fenu S, Travaglini L, Finolezzi E, Mancini M *et al.* Sequential Valproic acid/all-*trans* retinoic acid treatment reprograms differentiation in refractory and high-risk acute myeloid leukemia. *Cancer Res* 2006; **66**: 8903–8911.
203 Bug G, Schwarz K, Schoch C, Kampfmann M, Henschler R, Hoelzer D *et al.* Effect of histone deacetylase inhibitor valproic acid on progenitor cells of acute myeloid leukemia. *Haematologica* 2007; **92**: 542–545.
204 Gore SD, Baylin S, Sugar E, Carraway H, Miller CB, Carducci M *et al.* Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res* 2006; **66**: 6361–6369.
205 Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, Yang H, Rosner G, Verstovsek S *et al.* Phase 1/2 study of the combination of 5-aza-2′-deoxycytidine with valproic acid in patients with leukemia. *Blood* 2006; **108**: 3271–3279.
206 Soriano AO, Yang H, Faderl S, Estrov Z, Giles F, Ravandi F *et al.* Safety and clinical activity of the combination of 5-azacytidine, valproic acid, and all-*trans* retinoic acid in acute myeloid leukemia and myelodysplastic syndrome. *Blood* 2007; **110**: 2302–2308.